Analysis of amoxicillin in counterfeit antibiotics from the Subcontinent and the Middle East

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Analysis of Amoxicillin In Counterfeit Antibiotics

From

The Subcontinent And The Middle East

By

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(M. Sc by Research in Pharmaceutical Analysis)

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Declaration:

I confirm that where otherwise indicated in the thesis, the work detailed including literature search and the experimental work is my own investigation. This research work has not been accepted or concurrently being submitted for any other degree at any institution. All material contained has been reviewed and the text is my own description. All copyrights reserved.

Mr. Muhammad Idrees

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Abstract:

The thesis comprises the study of counterfeit drugs in the Middle East and Subcontinent. An extensive literature review was carried out about counterfeit drugs across the world, consequences of counterfeit drugs and study about various strategies combating counterfeiting.

The thesis concerns the research project aiming the analysis of amoxicillin in counterfeit antibiotics. Various techniques used for the analysis of amoxicillin such as High Performance Liquid Chromatography, Capillary Electrophoresis and Infrared Spectroscopy were also studied in detail for developing ideas and to approach scientifically towards the research on counterfeit drugs.

A suitable method was developed for the CE analysis of amoxicillin in order to achieve better analysis i.e. accurate and repeatable. Amoxicillin was analysed using HPLC and CE and then the results were further confirmed using FT-IR technique.

The results obtained using HPLC and CE are comparable and suggest that almost all the different brands of amoxicillin (excluding sample 10) contained the active ingredients more or less the amount stated by the manufacturers and did not show counterfeiting. Different brands of amoxicillin were found to contain active ingredients within the internationally acceptable limits i.e. a sample tablet or capsule of 500 mg should not contain less than 90 % of active ingredients of the stated amount and similarly no sample should contain more than 120 % of the stated amount (United States's Pharmacopoeia). Although all the samples show repeatability within the same sample capsule, still a variation in content uniformity is observed between different sample capsules within the same brand of amoxicillin.

The comparison of results obtained from the analysis of all different brands of amoxicillin show the difference of quantity of amoxicillin found in the sample capsules of the same weight between the different brands but it also show the difference between results obtained for different capsules of the same brand (variation of content uniformity within a particular brand).

Sample 10 was highly suspected to be a counterfeit sample as one of the sample capsules (sample capsule 10a) did not contain any amoxicillin at all. These results were confirmed when sample capsule 10a was repeatedly analysed using HPLC and CE. The results produced by sample capsule 10a after each analysis on HPLC and CE were the same such that no peak was found for sample 10a after several repeated analysis using HPLC and CE techniques.

Further analysis of a 4^{th} capsule (capsule 10d) from sample confirmed that it contained almost the same amount of amoxicillin as stated by the manufacturer. Hence, this analysis suggested that the manufacturer of sample 10 might have produced the antibiotic meeting the international standards of quality control and the counterfeiting might have come from any point along the distribution chain of this product.

Glossary of Terms:

CE: Capillary Electrophoresis

HPLC: High Performance Liquid Chromatography

FT – IR: Fourier Transform Infrared

ATR-IR: Attenuated total reflectance Infrared

DRIFTS: DRIFTS Diffuse Reflectance Infrared Fourier Transform Spectroscopy

TLC: Thin Layer Chromatography

MeOH: Methanol

RFI: Radio Frequency Identification

RFID: Radio Frequency Identification Data

DNA: Deoxyribonucleic Acid

RNA: Ribonucleic Acid

SDS: Sodium Dodecyl Sulphate

UV: Ultra Violet

List of Contents:

Chapter 1: "Introduction"

This chapter comprises the aims of project, introduction and explanation of counterfeiting, antibiotics, properties and classifications of antibiotics and their various applications. The chapter also includes the theory of the analytical techniques which were used for the analysis of the antibiotic Amoxicillin in this work.

1.1. Aim and objectives:

Counterfeiting or the production of fake drugs is nowadays one of the most serious and threatening issues around the world, particularly in the developing and poor countries. The problem of counterfeiting is most prominent in Asian countries and can be found in a few developed countries as well. Before starting this research project, an extensive literature review was carried out and the life threatening problem of counterfeiting was studied in details.

The aim of the project was the analysis of amoxicillin in counterfeit antibiotics from the Middle East and Subcontinent and the project had the following objectives.

1. The collection of antibiotics from different companies of the Middle East and Subcontinent and analyse them quantitatively and qualitatively.

2. To establish new methods of analysis for antibiotic amoxicillin if possible by using HPLC and CE technique.

The initial HPLC and capillary electrophoresis analysis was performed through the quantitative analysis of some common pain killers such as aspirin, caffeine and paracetamol. The aim of analysing these compounds was to gain practical knowledge for the use of these modern analytical tools used in this research.

Once the analysis of aspirin, caffeine and paracetamol was successfully carried out, the analysis of different brands of amoxicillin was then carried out for the purpose of finding counterfeiting if any.

1.2. Counterfeit drugs:

A counterfeit drug or medicine is defined as a medication which is deliberately and fraudulently mislabeled with respect to identity, composition and source (Newton. P.N, et al: 2006 Lancet, 6. 602-610).

They are produced and sold in the market with the intent to deceptively represent the origin, authenticity or effectiveness of the original drug. The definition of counterfeit or fake drugs includes not only completely fake drugs but also those drugs that have been diluted, tampered with, adulterated, repackaged or relabeled so as to misrepresent the dosage, expiration dates and origin. These drugs are also known as substandard drugs and are cheaply produced in order to make unlawful profit. These medicines are sold in the market by labeling them as of the same standard as set by the local drugs regulatory authorities.

The counterfeit drugs are likely to contain insufficient quantity of active ingredients, or may contain entirely incorrect active ingredients (contaminants) (which may or may not be harmful), and which are typically sold with fake labeling and fake packaging.

Counterfeit drugs can generally be classified by the following characteristics:

- Re-labeled drugs that were previously expired, defective or otherwise deemed unfit for use.
- Re-labeled drugs wherein the active ingredient is fraudulently diluted.
- Re-labeled drugs wherein the active ingredient is adulterated or substituted.
- Falsely-labeled substances whose combined active ingredients meet one or more of the preceding criteria.

1.2.1. Consequences of counterfeit drug application:

The extent of consequences caused by counterfeit drugs is unknown. However, individuals on counterfeit medication may experience a number of dangerous consequences to their health, such as allergic reactions, unexpected side effects, or a worsening of their medical condition. Some times the counterfeit drugs may be without any active ingredients, and may contain inert substances, which may not provide the patients any treatment benefits.

The problem of counterfeiting is difficult to detect, investigate, and quantify. It simply means that it is hard to know or even estimate the true extent of the problem. It is estimated that counterfeit drugs account for nearly 1 % to 50 % of the worldwide trade in pharmaceutical industry (Newton. P.N, et al: 2006 Lancet, 6. 602-610).

Counterfeit drugs are more likely to be introduced as a part of drug distribution process involving multiple wholesalers. In the current distribution system, products are repackaged for several legitimate reasons, such as to improve efficiencies for automated systems. However, in Europe, the products are packaged in quantities that relate to a course of treatment (unit of use,) thereby avoiding the need for repackaging.

The problem of counterfeiting is most threatening in the developing world. It has been found that these substandard drugs are available in the markets across the developed world as well but the problem is more severe in developing world as compared to the developed countries like the UK and USA (Charatan: 2001 British Medical Journal, 322-144).

In the developing countries, the counterfeiting is found to be targeting the drugs which are used in high volumes and are used for the treatment of life threatening diseases. These drugs mainly include antibiotics, anti-malarial drugs, drugs used for the treatment of heart diseases, anti-viral drugs, anti-cancer drugs and the

drugs used for the treatment of HIV/AIDS and TB (Khan, et al: 2007 Indian J. Pharmacol, 39. 206 - 207).

According to the estimation made by the US FDA across Asia is that the trade in fake drugs is from $35 billion to $45 billion, as many as half of the drugs are sold to be believed substandard are fake drugs. These include drugs for AIDS, tuberculosis, antibiotics and even meningitis vaccines and anti-malarial drugs as well (Khan, et al: 2007 Indian J. Pharmacol, 39. 206 - 207).

According to the UK's Daily Telegraph report (August 2005), as many as 100,000 people die each year in China as a result of taking counterfeit or fake drugs. These figures indicate that the counterfeit drugs are found in high ratio in china as compared to other neighboring countries (World Pharmaceutical Frontiers: 2008 The Cost of a Cure).

In Pakistan, the problem of counterfeiting is threatening the public health on a large scale. These drugs are sold in pharmacies and small shops in the market under fake labeling and packaging.

These counterfeit drugs are always sold with names similar to the original products. The problem of counterfeiting is most prominent in small cities and towns where there is a lack of education and the people are mostly illiterate.

The drug controlling authorities in Pakistan are taking strict and positive steps to defeat the problem of counterfeiting in Pakistan. The law enforcement agencies are also working together with drug monitoring agency to uproot this threat of counterfeiting.

1.2.2. Combating counterfeiting in Asia with the help of new technologies and plans.

There are several technologies that may prove helpful in combating the problem

of counterfeiting, such as radiofrequency identification, which uses electronic devices to track and identify pharmaceutical products, by assigning individual serial numbers to the containers holding each product.

As stated earlier, counterfeit drugs are a threatening and dangerous problem in Asia. In most of the Asian countries, about half of the total drugs produced are counterfeit drugs. Poor public health conditions and high prices of drugs have provided a chance to the industries producing counterfeit drugs and selling them on cheap prices. As a result in some Asian countries, the governments and pharmaceutical industries have begun to struggle more combating the problem of counterfeiting.

Some pharmaceutical companies including GlaxoSmithKline, Merck and Knoll pharmaceuticals have implemented special type of holograms on their products in order to differentiate them from counterfeit or substandard types.

The Malaysian ministry of health has also introduced such holograms on their pharmaceutical products as a security measure. These holograms are tagged and registered with Meditag, a security device. Each Meditag has a unique serial number and can be scanned with a special device to verify its authenticity. However, these holograms are not too much dependable as it is possible to copy holograms. Hence they are not sufficient to stop counterfeiting (Pacific Bridge Medical: 2006 Asian Medical eNewsletter).

Another technique is radio frequency identification or RFI which contain a chip with an antenna is affixed to the drug packaging. The exact location of the drug can then be found by radio waves. This technology is not cheap but is said to be one of the most effective techniques developed so far to uproot and stop counterfeiting. Some pharmaceutical companies such as Purdue Pharma and Pfizer have already started RFID technology to trace their respective products, OxyContin and Viagra (Pacific Bridge Medical: 2006 Asian Medical eNewsletter).

In order to combat counterfeiting, the pharmaceutical scientists are looking to produce drugs through reaction mechanisms comprising of several step reactions rather than simple and short reaction mechanisms. The reason for following long and complicated reaction mechanisms is to make the production of life saving drugs more expensive and complicated so that the counterfeiting industries are unable to produce the life saving drugs.

1.3. Antibacterial Antibiotics:

Historical Background

The historical background of antibiotics can be summarised as follows

- As early as 500 to 600 B.C., molded curd was used in medicines by Chinese to treat boils and carbuncles.
- Chinese also used molded cheese to treat infected wounds which is assumed to possess antibiotic substance.
- Sir Alexander Fleming discovered the antibacterial properties of penicillin in 1929 (Microbiology Procedures: 2007 Antibiotics).

In 1877 Pasteur and Joubert discovered that anthrax bacilli were killed when grown in a culture in the presence of certain bacteria (Microbiology Procedures: 2007 Antibiotics).

- Later on Florey and Chain introduced amoxicillin into therapy thus begun the practical medical use of antibiotics.

Definition of antibiotics

Antibiotics were defined in different ways by different people in early days. Some of the definitions are given as follows

1. Vuillemin to define antibiotics (literally, against life") as the biologic concept of survival of the fittest, in which one organism destroys another to preserve itself.

2. According to Waksman, “an antibiotic or antibiotic substance is produced by microorganisms, which has the capacity of inhabiting the growth or even of destroying other microorganisms” (Microbiology Procedures: 2007 Antibiotics).

Later proposals have sought both to expand and to restrict the definition to include any substance produced by a living organism that is capable of inhabiting the growth or survival of one or more species of microorganisms in low concentrations.

Later on, naturally occurring antibiotics were modified into synthetic antibiotics. This advancement permits to define a substance as an antibiotic if it meets the following conditions.

- It is a product of metabolism (although it may be duplicated or even have been anticipated by chemical synthesis).
- It is a synthetic product produced as a structural analogue of a naturally occurring antibiotic.
- It antagonises the growth or survival of one or more species of micro-organisms.
- It is effective in low concentration.

Applications of antibiotics:

The successful use of antibiotics in the treatment of human diseases has prompted the expansion of their use in a number of related fields. Extensive use of their antimicrobial power is made in veterinary medicine.

The discovery that low-level antibiotic administration to meat-producing animals resulted in faster growth, lower mortality and better quality, has led to the use of these products as food supplements. Several antibiotics are used in the treatment of many bacterial and fungal diseases of plants.

Their use in food preservation is being studied carefully. Indeed such uses of antibiotics have made necessary careful studies of their long term effects on humans and their effects on various commercial processes (Gurdeep, R. Chatarwal, and Sham Anand: 2000. 5th).

For example, foods containing low-level of antibiotics may cause allergic reactions in hypersensitive persons. Similarly the presence of antibiotics in milk may interfere with the formation of cheese.

The success of antibiotics in therapy and in related fields has made them one of the most important products of the drug industry today.

Production of Antibiotics:

The commercial production for medical use follows a general pattern, differing in details for each antibiotic. The general pattern may be divided in to six steps (Herbarium USU, 2007 Production of Antibiotics).

1. Preparation of a pure culture of the desired organism for use in the inoculation of the fermentation medium.
2. Fermentation during which antibiotic is formed.
3. Isolation of the antibiotic from that culture medium.
4. Purification.
5. Assays for potency, sterility, absence of pyrogens, and other necessary data.
6. formulation into an acceptable and stable dosage form

Spectrum of activity:

The ability of some antibiotics such as chloramphenicol and tetracyclines, to antagonise the growth of numerous pathogens has resulted in their being designated as, “broad spectrum” antibiotics. Designations of the spectrum of activities are of somewhat limited use to a physician unless they are based on

the clinical effectiveness of the antibiotic against specific micro-organisms.
Many of the broad spectrum antibiotics are effective only in high concentrations against some of the species of micro-organisms often included in the spectrum.

Reaction mechanism of antibiotics:

The manner in which antibiotics act against microorganisms is varied. The mechanisms of reactions of some of the more common antibiotics are listed in the form of tables depending on the site of actions as shown in Tables 1, 1.1, 1.2 and 1.3.

Table.1: The reaction mechanism of antibiotics the site of action being cell wall.

Site of Action	**Antibiotic**	**Process Interrupted**	**Type of Activity**
Cell Wall	Bacitracins	Mucopeptide synthesis	Bactericidal
	Cephalosporins	Cell wall cross linkage	Bactericidal
	Penicillin e.g. Amoxicillin	Cell wall cross linkage	Bactericidal

Table.1.1: Reaction mechanism of antibiotics the site of action being cell membrane.

Site of Action	**Antibiotic**	**Process Interrupted**	**Type of Activity**
Cell membrane	Amphotericin B	Membrane function	Fungicidal
	Nystatin	Membrane function	Fungicidal
	Polymericin	Membrane integrity	Bactericidal

Table.1.2: Reaction mechanism of antibiotics, the site of action being the Ribosomes.

Site of Action	Antibiotic	Process Interrupted	Type of Activity
Ribosomes	Chloramphenicol	Protein synthesis	Bactericidal
50s sub units	Erythromycin	Protein synthesis	Bactericidal
30s sub units	Aminoglycosides	Protein synthesis and fidelity	Bactericidal

Table.1.3: Reaction mechanism of antibiotics, the site of action being the nucleic acids.

Site of Action	Antibiotic	Process Interrupted	Type of Activity
Nucleic acids	Actinomycin	DNA and RNA synthesis	Panicidal
	Griseofulvin	Cell division and micro tubules assembly	Fungistatic
DNA or RNA	Mitomycin C	DNA synthesis	Panicidal
	Refampin	mRNA	Bactericidal

In many instances the mechanism of action is not fully known, for a few (e.g. penicillins) the site of action is known but precise details of the mechanism are still under consideration.

The mechanism of action of an antibiotic determines, in general, whether the agent exerts a -cidal (killing) or static (holding) action. The distinction may be

important for the treatment of serious life-threatening infections particularly if the natural defense system of the host is either deficient or overwhelmed by infection.

In such a case a -cidal agent is obviously indicated but much work is to be done in this area and as mechanisms of actions are revealed, the development of improved structural analogues of effective antibiotics will continue to increase.

1.4. β-Lactam antibiotics (the class of amoxicillin):

Antibiotics which contain the β-lactam (a four membered cyclic amide) ring structure constitute the dominant class of antibiotic agent currently employed for the chemotherapy of bacterial infections (Gurdeep, R. Chatarwal, and Sham Anand: 2000. 5th).

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The first antibiotic to be used in therapy of bacterial diseases was penicillin (penicillin G or benzyl penicillin) and a close biosynthetic relative phenoxymethyl penicillin remain the agents of choice for the treatment of infections caused by most species of Gram positive bacteria.

The discovery of a second group of β-lactam antibiotic the cephalosporins and the chemical modification of naturally occurring penicillins and cephalosporins have provided semi-synthetic derivatives that are variously effective against bacterial species known to be resistant to penicillin, in particular, penicillinase producing staphylococci and Gram negative bacilli.

Hence, apart from a few resistant species that have either inherent or acquired resistance, almost all bacterial species are sensitive to one or more of the available β-lactam antibiotics.

1.4.1. The Penicillins:

Production of Penicillins:

Until 1944, it was assumed that the active principal in penicillin was a single substance and that the variation in the activity of different products was due to the amount of inert material present in the sample (Gurdeep, R. Chatarwal, and Sham Anand: 2000. 5th).

Now, it is known that during the biologic elaboration of antibiotics, several closely related compounds may be produced. These compounds differ chemically in acid moiety of the amide side chain. The difference in this moiety produces differences in the antibiotic activities of the various agents. This variation in moiety also affects the physicochemical properties. Thus it has become proper to speak of penicillins as a group of compounds and to identify each of penicillins specifically. As each of the different penicillins was first isolated letter designations were used in the United States; the British used Roman numerals**.**

Many of the different kinds of penicillins have been isolated from fermentation mixtures. Some of these occur naturally while others have been biosynthesized by altering the cultural medium to provide certain be incorporated as acyl groups. The commercial production of biosynthetic penicillins today mainly depends on various strains of penicillium notatum and p-chrysogenum. In recent years, many more penicillins have been prepared semi-synthetically and undoubtedly many more will be added to the list to find more effective and valuable products.

The commercial production of penicillin has increased markedly since its introduction. As its production increased, the cost dropped correspondingly. When penicillin became available for the first time, its 100,000 units were sold for 20 dollars (Gurdeep, R. Chatarwal, and Sham Anand 2000: 5th).

Fluctuations in the production of penicillin throughout the years have reflected the following changes.

- The popularity of broad spectrum antibiotics compared with penicillins
- The development of penicillin resistant strains of several pathogens
- The more recent introduction of semi-synthetic penicillin
- The use of penicillins in animal foods and for veterinary purposes and
- The increase in marketing problems in a highly competitive sales area.

Allergic reactions to penicillins:

Allergic reactions to various penicillins, ranging in severity and may include

- A variety of skin and mucous membrane rashes
- Drug fever (fever caused by a drug due to its allergic reaction)
- Anaphylaxis (an acute multi-system allergic reaction)

The penicillins most frequently implicated as the cause of allergic reactions are penicillin G and ampicillin. However, virtually all commercially available penicillins have been reported to cause such reactions.

Classification of Penicillins:

A number of designations have been used for classifying penicillins, based on their sources, chemistry, pharmacokinetic properties, resistance to enzymatic spectrum of activity and clinical uses. Therefore, keeping in view these designations a simple form of classification can be given in the table 1.4 on the following page.

Table.1.4: Classification and properties of penicillins:

Penicillin	**Source**	**Acid resistance**	**β-lactamase Resistance**	**Spectrum of Activity**	**Clinical use**
Benzyl penicillin	Biosynthetic	Poor	No	intermediate	multi-purpose
Phenoxymethyl Penicillin	Biosynthetic	Good	No	intermediate	multi-purpose
Methicillin	semi-synthetic	Poor	yes	Narrow	Limited
Oxacillin	semi-synthetic	Good	yes	Narrow	Limited
Dicloxacillin	semi-synthetic	Good	yes	Narrow	Limited
Ampicillin	semi-synthetic	Good	no	Broad	multi-purpose
Amoxicillin	semi-synthetic	Good	no	Broad	multi-purpose
Peperacillin	semi-synthetic	Poor	no	Extended	Limited

1.5. Amoxicillin:

Amoxicillin is a semi-synthetic Penicillin, introduced in 1974 and is simply the p-hydroxy analogue of Ampicillin (Sciencedirect 20007). It is prepared by the acylation of 6-APA (6-aminopenicillanic) with p-hydroxyphenylglycin.

It is an antibiotic with a broad spectrum of bacterial activity that is used against many of the Gram-positive and few Gram-negative bacteria. Its antibacterial

spectrum is nearly identical to that of Ampicillin, and like Ampicillin, it is resistant to acids, susceptible to alkaline and β-lactamase hydrolysis and is weakly protein bound.

Mode of action:

It acts against bacterial activity by inhibiting the synthesis of bacterial cell walls. Amoxicillin stops the cross-linkage between the linear peptidoglycan polymer chains that make up a major component of the bacterial cell wall.

Physical Characteristics of Amoxicillin:

- It is a white crystalline powder
- Practically, odorless and tasteless.
- It is sparingly soluble in water
- Practically insoluble in ether, chloroform and fixed oils.
- It dissolves in dilute solutions of alkali hydroxides.

Microbiology:

Amoxicillin is a moderate-spectrum antibiotic active against a wide range of

1. Gram-positive (Streptococcus spp, Non-β-lactamase producing Staphylococcus spp and Streptococcus faecalis). And
2. Limited range of Gram negative bacteria (Haemophilus influenzae, Neisseria gonorrhoeae, Neisseria meningitides etc.).

Resistant organisms:

There are some microorganisms which offer resistance to the action of Amoxicillin such as penicillinase producing organisms (particularly penicillinase producing staphylococcus spp etc).

However, Amoxicillin has been shown to eradicate the resistant organisms if its routinely given concentration (in pediatrics) is doubled (Red Book, 2003 Report of the Committee on infectious diseases, American Academy of Pediatrics).

Formulation:

Amoxicillin is available in tri-hydrate form as capsules and syrups for oral use and in the form of the sodium salt for intravenous administration.

Advantages of Amoxicillin over Ampicillin:

Orally administrated Amoxicillin has some advantages over Ampicillin such as

- More complete gastrointestinal absorption to give higher plasma and urine levels
- Less diarrhea
- Little or no effect of food on absorption.

Thus amoxicillin has largely replaced ampicillin. However, amoxicillin is reported to be less effective than ampicillin in the treatment of bacillary dysentery, presumably because of its greater gastrointestinal absorption.

Figure.1. Structure of Amoxicillin:

Chapter 2: Theory of Analytical Techniques:

The theory of analytical techniques such as High Performance Liquid Chromatography, Capillary Electrophoresis, Infrared Spectroscopy and Thin Layer Chromatography used for the analysis of amoxicillin will be discussed in this chapter.

2.1. High Performance Liquid Chromatography (HPLC):

High performance liquid chromatography is an analytical technique used for the separation and determination of organic and inorganic solutes in any sample especially biological, pharmaceutical, food, environmental, industrial, etc.

In the process of chromatography a liquid permeates through a porous solid stationary phase and elutes the solutes into a flow-through detector. The stationary phase is usually in the form small uniform particles with a diameter ranging from 5-10µm, packed into a cylindrical column. The typical column is made of a rigid material such as stainless steel or plastic and is generally 5-30 cm long having internal diameter ranging from 1-9mm.

2.1.1. HPLC System:

In a typical HPLC system, a detector measures response changes between the solvent itself, and the solvent and sample electrical response is digitized and sent to a data system. An HPLC system comprises of the following parts.

1. **Solvent delivery system.** It pushes the solvent stream through the instrument at a constant flow rate.
2. **Column.** A stainless steel tube packed with silicon beads that separate the desired compound from the other compounds.
3. **Auto-sampler.** It introduces the sample into the main liquid stream of the system.
4. **Detector.** An optical sensor is fixed in the HPLC system that detects the changes in the characteristics of the solvent stream.

5. **Data system.** There is always a data system used as a means of controlling the system components and storing, processing and displaying the data.

A high pressure is also fixed in the HPLC device to push the mobile phase through the column at a typical flow rate ranging from 0.1 to 2ml/min.The sample to be separated is introduced into the mobile phase system by an injection device, manual or automatic, prior to starting the analysis. A schematic diagram of HPLC system is shown in Figure 1.1.

Figure.1.1: Various parts of HPLC system.

Types of HPLC based on force of attraction:

There are three main types of HPLC based on forces of attraction found between analyte, stationary phase and mobile phase. These types are

- Charge based HPLC
- Reverse phase HPLC
- Normal phase HPLC

Charge based HPLC is based on the forces of attraction found between oppositely charged particles. Charge based HPLC is of two types

a) Ion pairing HPLC which involves smaller molecules
b) Ion-exchange HPLC which involves relatively larger particles and the charge particles bend reversibly towards the sample molecules.

In charge based HPLC, the process of desorption is carried out by increasing the salt concentration or changing the pH of the mobile phase. Charges involving charge based HPLC are

- Inorganic anion such as Cl^-, Br^- and SO_4^{-2}.
- Inorganic cations
- Organic acids
- Organic bases

Reversed phase HPLC involves the use of a non-polar stationary phase and a slightly polar mobile phase. It does not involve any ionic forces of attraction or any charged particles. The retention time for non-polar molecules is longer as compared for polar molecules having less retention time. The reversed phase HPLC works on the principle of interactions between relatively polar solvents, non-polar solvent and non-polar stationary phase.

The third and simplest type of HPLC is the normal phase HPLC. This type of HPLC involves the use of a polar stationary phase and a non-polar mobile phase.

Types of HPLC based on its Applications:

HPLC has the following main types based on its applications.

Preparative HPLC. This type of HPLC involves the isolation and purification of compounds. It provides information about the compound produced in the process of preparation. It is also used to obtain various informations such as identification, quantification and resolution of compounds.

Type of HPLC based on chemical separation. This type of HPLC involves the separation of various chemicals using the difference between the migration times of compounds. This can be achieved by using suitable mobile phase and columns.

HPLC based on purification. This type of HPLC involves the separation or

isolation of a desired or targeted compound from a mixture of other compounds or contaminants. Each substance produces a characteristic peak on HPLC apparatus which is used as a basis for purification of compounds.

HPLC based on identification of compounds. This type of HPLC is set up identify a given compound or to identify each and every single compound in a mixture of compounds. The identification process needs suitable detectors and then the development of a separation assay. The desired peak produced for the compound to be identified should have the following characteristics.

a. Reasonable retention time
b. Repeatability of retention time and
c. Well defined and separated from the other peaks.

HPLC based on quantification. This type oh HPLC involves the determination of unknown concentration of a compound in known solution of known concentration. The process of quantification could be more easily understood by the calculations in the coming chapters.

Current Developments:

HPLC is one of the most useful lab-separation techniques used by analytical chemists. It is of enormous use not only these days but its present importance shows that this technique will gain more popularity and will further add to the ease of separation chromatography. It is more obvious that in the near future, HPLC will be used in the field of biotechnology and life sciences. LC-MS is another separation technique and is a combination of liquid chromatography and mass spectrometry. Biotechnology companies and pharmaceutical industries will be using HPLC for two main reasons.

a) To assure the government and public that the products the produce are non-toxic
b) The products they produce are always in their pure forms.

Furthermore, fast and micro bore columns will be used in pharmaceutical industries, biotechnology and environmental sciences to reduce the time period analysis. The light scattering detectors may replace the RI detectors in the near future. Another possibility is the use of robots to analyse hazardous samples such as AIDS, viral or bacterial samples, radio active isotopes or environmental contaminants.

2.2. Advantages of HPLC:

1. HPLC offers a higher resolution and greater speed of analysis.
2. Greater reproducibility (+/- 1%) because of close control of the parameters affecting the efficiency of the separation.
3. High sensitivity [ranging from nano gm (10^{-9}) to femto gm (10^{-15})]
4. It offers an easy automation of instrument operation and data analysis.
5. HPLC columns can be reused without repacking or regeneration.
6. Adaptability to large-scale, preparative procedures.

2.2.1. Disadvantages of HPLC:

1. HPLC is more expensive as compared to other analytical techniques.
2. It is a complicated technique, this reason is one of it’s main demerit.
3. HPLC shows low sensitivity towards the analysis of certain compounds.
4. Irreversibly absorbed compounds cannot be detected.
5. And co-elution of the compound is very difficult to detect.

2.3. The Capillary Electrophoresis (CE):

The process of electrophoresis is defined as “the differential movement or migration of ions by attraction or repulsion in an electric field.
In practical terms, a positive (anode) and a negative (cathode) electrode are placed in a solution containing ions. Afterwards, voltage is applied across the electrodes, solute ions of different charges, such as anions which are the negatively charged ions and cation (positively charged ions) start moving through the solution towards the oppositely charged electrodes.

Hence the capillary electrophoresis is the technique of performing electrophoresis in buffer filled narrow bored capillaries, normally ranging from 25 to 100 micrometers in internal diameter.

In its classical form, it is used to separate the mixtures of charged solute species by differential migration through a buffered electrolyte solution supported by a thin slab a short column of a polymeric gel such as polyacrylamide or agarose, under the influence of an applied electric field that creates a potential gradient.

Two platinum electrodes are placed in an electrolyte which is contained in a reservoir at opposite ends of the supporting medium and then the electrodes are connected to an external DC power supply.

A considerable amount of heat may be produced during the separation of components of a mixture at a higher applied voltage. To stabilize the temperature, many systems use water-cooling. As the process is carried out under the effect of an external applied electric field, the buffer solution undergoes electrolysis, a producing hydrogen and oxygen at cathode and anode respectively, and the have to be replenished or the buffer has to be renewed to maintain pH stability.

Cationic solute species that are positively charged species migrate towards the

cathode and the anionic species having negative charge migrate towards anode, while the neutral species do not move at all and remain close to the point at which the sample was introduced to the system.

2.3.1. Instrumentation of CE:

The CE device is very simple in design. It features the following main parts.

Injector: The first part of a CE device is the injector. It is used to introduce the sample into the capillary and may be accomplished using pressure or voltage.

Capillary: Second part of a CE apparatus is narrow bore capillary. It is typically made of fused silica and can be fabricated from borosilicate as well. The surface charge of a capillary is important in affecting the electro-osmotic flow.

Power Supply: Third part of the CE is a high voltage power supply. This source of power supply is used to apply voltage to cathodic or anodic reservoir when the reservoirs are grounded. A platinum electrode is used to connect the high voltage source to the capillary electrophoresis running buffer because of its relative inertness.

Detector: Fourth part of the CE apparatus is a detector which is one of the most important parts of a CE apparatus. CE can be connected to a number of detection devices. Some of the most common detection devices are UV visible absorbance detection, laser induced fluorescence, mass spectrometry and electrochemical detection. UV absorbance detection is the most common and nearly universal technique used in CE analysis.

Computer: Fifth part of the CE device is a computer. It is used to convert analog data output from the detector to digital format for software analysis. The software packages used to control the CE systems are mostly based on the existing HPLC software.

The sample is injected onto the capillary by temporarily replacing one of the buffer reservoirs (normally at the anode) with a sample reservoir and applying either an electric potential or external pressure for a few seconds.

After replacing the buffer reservoirs, an electric potential is applied and hence the process of separation is performed. Optical (UV-visible or fluorometric) detection of the separated components of the analyte can be achieved directly through the capillary wall near the opposite end (normally near the cathode).

Figure1.2: shows a typical capillary electrophoresis instrument.

2.3.2. Theory of capillary electrophoresis:

The theory of capillary electrophoresis explains the process of CE very successfully and is therefore, applied to CE device and can be show in the form of following equations.

As the electrophoresis is the movement or migration of ions or solutes under the effect of an applied electric potential therefore, the separation depends on the migration velocities of ions or solutes to be separated and can be given as

$$\upsilon = \mu_e E \qquad \text{(a)}$$

Where υ is ion migration velocity in m / sec, μ_e is electrophoretic mobility in metre squared / V sec, and E is the electric field strength in V / m.

This expression shows a balance of two opposite forces acting on the ion. These forces are,

- Electrical force which favours the motion of ion
- Force of fraction which opposes the motion of ion

The electrophoretic mobility is always constant as the forces acting on the moving ion balance each other, hence

$$\mu = q / 6 \pi \eta r \qquad (b)$$

Where η is the viscosity of the buffer, π is a constant with a value of 3.14, q is the charge on the ion and r is the ion radius.

This equation shows a relation between charge to mass ratio of an ion and the electrophoretic mobility. Charge q is always fixed for fully dissociated ions such as strong acids and small ions and can be affected by the pH alterations.

Electro osmotic flow:

The bulk flow of a liquid through a capillary under the influence of an applied electric force is called the electro-osmotic flow which is caused as follows.

When the capillary of a CE is filled with buffer, it causes the following phenomena to take place.

1. Negatively charge walls attract positive ions from the solution
2. It causes a potential difference close to the capillary walls called zeta potential.

According to the Stern model of electric double layer (consisting of rigid layer of adsorbed ions and a diffused layer), in which ion diffusion may occur due to thermal motion. When a voltage is applied across the capillary, cations in the diffused layer are free to migrate towards the cathode, carrying the bulk solution with them. The result is the net flow in the direction of the cathode, with a certain velocity given as under

$$\upsilon_{EOF} = (\varepsilon_O \varepsilon \zeta / 4\pi\eta) \qquad (c)$$

Where ε_O is the dielectric constant of a vacuum,ε is the dielectric constant of the

buffer, ξ is the zeta potential, η is the viscosity of the buffer and E is the applied electric field. The terms enclosed in the brackets equate to the mobility of the EOF.

The relationship between EOF mobility and EOF velocity is analogous to that between electrophoretic mobility and migration velocity. And also the units for EOF are the same as for the electrophoretic mobility.

Applications of Capillary Electrophoresis:

1. Pharmaceutical applications. Pharmaceutical industry uses capillary electrophoresis for a number of reasons and applies the CE technique to the assay of drugs, determination of drugs related impurities, analysis of small molecules and ions and the analysis of pharmaceutical products.

Pharmaceutical industry uses capillary electrophoresis because of its low cost, reduced solvent consumption and disposal, and fast and speedy analysis.

2. Food analysis. Capillary electrophoresis is used in the food analysis to maintain a high quality and known composition and for the detection of any contaminants if present.
3. Chiral analysis. Keeping in view the chiral selectors, CE is applied to stereo selective analysis, soluble and chemically stable compounds analysis and efficient analysis of compounds with respect to their rapid complextaion kinetics.
4. Analysis of vitamins. Capillary electrophoresis is successfully applied to the analysis of some vitamins as well.
5. CE applied to environmental analysis. Capillary electrophoresis is newly introduced technique to environmental analysis. Capillary electrophoresis is used in environmental science for the analysis for the analysis of toxic and hazardous samples from waste sites.

Capillary electrophoresis is used in analytical chemistry for polar volatile compounds, semi volatile and non-volatile compounds, inorganic cations and anions as well.

2.4. The infrared spectroscopy:

It is one of the most useful and powerful techniques offering the possibility of chemical identification in analytical chemistry. It refers to the part of electromagnetic spectrum between the visible and microwave region.

The most useful advantage of IR is that it is used to for the structure elucidation of molecules. The infrared spectroscopy is based on the fact that the sample under observation shows a well defined absorption in the IR region.

The electromagnetic spectrum has waves of different length exhibiting different frequencies, therefore a possible relation between the wave length and frequency of these waves can be given as under

$$\nu = c/\lambda \quad \text{and} \quad \lambda = c/\nu \qquad a$$

Where ν is the frequency of waves (number of wave cycles that pass through a point in one second). It is measured in Hertz, where 1 Hz = 1cycle/sec.
λ is the wave length of one complete wave cycle and is often measured in cm. where c is the speed of light and is always a constant.
Hence if c = constant then from equation a

$$\nu = \text{constant}/\lambda \quad \text{or}$$
$$\nu \propto 1/\lambda \quad \text{or}$$
$$\lambda \propto 1/\nu \quad (c = \text{constant}) \qquad b$$

Wave length λ and frequency ν are inversely related to each other, shorter the wave length higher the frequency and longer the wave length lower will be the frequency.
Similarly, the energy E of the wave is related to the wave length λ and frequency ν by the following equations:

$$E = h\nu \qquad c$$

Where h is a constant called Plank's constant and ν is the frequency of the wave having energy E, but as we know that

$\nu = c/\lambda$ from equation a hence by putting the value of frequency from equation a in equation c we get

$$E = hc/\lambda \qquad d$$

These two equations (c and d) show relationship between energy and frequency, and energy and wave length respectively.

Equation c shows that energy E is directly related to the frequency of the waves, and from equation d, it is clear that energy E is inversely proportional to the wave length λ as h is constant in both cases.

$E = \text{constant } \nu$ or

$E \propto \nu$ similarly

$E = \text{constant } c/\lambda$

As c = constant as well then

$E = \text{constant}/\lambda$ or

$E \propto 1/\lambda$

That shows the relationship between energy, frequency and wave length.

Different subdivisions of IR regions:

Infrared region is further subdivided into the following regions

- The near IR region also known as vibrational rotation region ranging from 14000 cm^{-1} to 4000 cm^{-1}

This region is usefully applied in pharmaceutical analysis for raw material testing, product quality control and process monitoring.

- The mid IR region ranging from 4000 cm $^{-1}$ to 400 cm $^{-1}$
- Far IR region ranging from 400 cm $^{-1}$ to 10 cm $^{-1}$

2.4.1. Bond stretching:

The atoms of covalently bonded molecules are not bonded through rigid bonds and the bonds are therefore, relatively flexible as the two nuclei are attracted by the same pair of electrons. Thus these nuclei can vibrate to and fro (backward and forward) towards and away from an average position.

The phenomenon of bond stretching can be simplified with the help of the following diagram showing the stretching that occurs in carbon oxygen single bond. There will, of course, be other atoms attached to both carbon and oxygen. For example it could be the carbon and oxygen bond in methanol, CH_3OH.

H_3C…….OH

H_3C……OH

H_3C…….OH

Figure .1.3: Vibrations due to bond stretching and contracting.

The energy involved in this vibration depends on factors such as length of the bond and the mass of the atoms at either end. The greater the bond length, the more easy will the vibration occur and vice versa.

2.4.2. Molecular vibrations:

The vibrations exhibited by molecules are called molecular vibrations. These vibrations are of two types.

1. Stretching vibrations
2. Bending vibrations

In routine life while studying chemistry, one may think the molecules having rigid

and fixed bonds with rigid bond angles. But the actual case is not like this and is different from these imaginations because the bond lengths and the angles represent the average position about which the atoms vibrate.

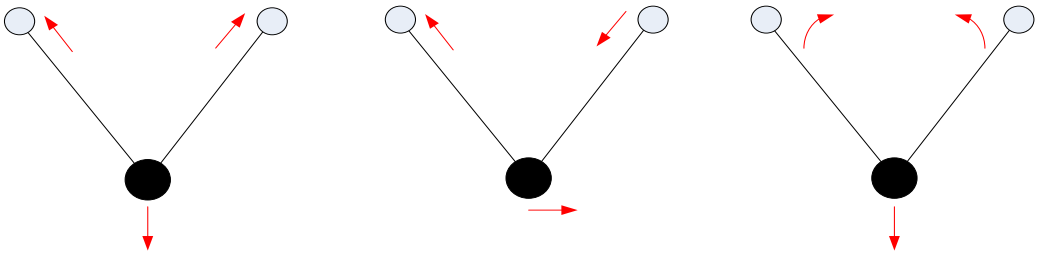
A non-linear molecule may have three rotational and two vibrational degrees of freedom. The remaining degrees of freedom correspond to fundamental vibrations. Similarly for linear molecules, there are two rotational and three translational degrees of freedom.

The net numbers of fundamental vibrations for linear and non-linear molecules are mentioned in the following table.

Table.1.5: Number of fundamental vibrations for linear and non-linear molecules.

Molecule	Degrees of freedom
non-linear	3n-6
Linear	3n-5

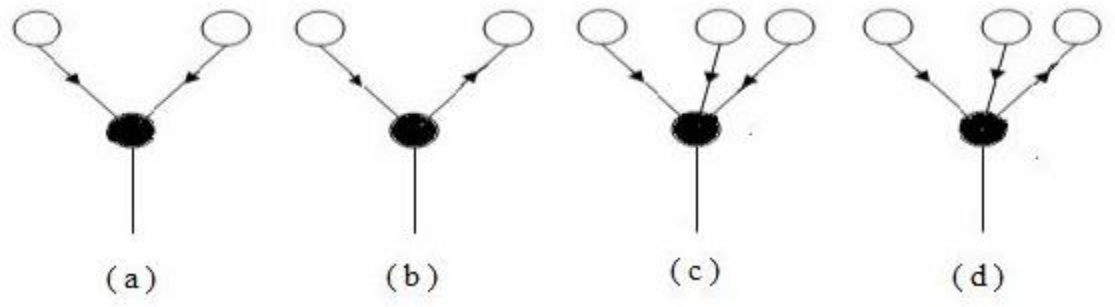
The fundamental vibrations of a molecule correspond to the number of bands in IR spectrum produced by that molecule (the actual number is some times different). The fundamental vibrations for water molecule (water is a linear molecule having three fundamental vibrations) are as under.



Symmetrical stretching asymmetrical stretching scissoring (bending)

Figure.1.4: Stretching and binding vibrational modes of H_2O.

Carbon dioxide, CO_2, is linear and hence has four fundamental vibrations as shown in the fig 4.3. Thus a symmetrical stretch of CO_2 gives a strong band in the IR at 235 $cm.^{-1}$The two scissoring and binding vibrations are equivalent and therefore, have the same frequency and therefore are said to be degenerate, appearing in the IR spectrum at 666 $cm.^{-1}$



a = Symmetric stretching vibration of molecule AB2

b = Asymmetric stretching vibration of the AB2 molecule.

c = Symmetric stretching vibration of molecule AB2.

d = Asymmetric stretching vibration of the AB2 molecule.

Figure.1.5: Stretching and bending vibrational modes for CO_2.

The symmetrical stretch of CO_2 is inactive in the IR because this vibration produces no change in the dipole moment of the molecule. In order to be IR active, a vibration must cause a change in the dipole moment of the molecule.

2.4.3. Instrumentation of IR.

The device used for IR analysis is called IR spectrometer. It has the following main parts.

1. Light source. It is usually a tungsten halogen lamp.
2. Detector. They are made of silicon, lead sulphide, Indium Gallium Arsenide.
3. Monochromator. Used to separate the poly chromatic IR spectral region into monochromatic frequencies.

Before using IR for any analysis, it should be calibrated involving the following steps.

1). Selection of representative calibration sample set.

2). Spectra acquisition and determination of reference values.

3). Multivariate modeling to relate the “spectral variations” to the reference values.

4). Validation of the model by cross validation, set validation or external validation.

Figure.1.6: Shows schematic representation of FT.IR instrument.

Applications of IR:

The infrared technique very useful technique and is applied to the analysis of a large number of compounds. Some of its applications are discussed as follows.

Combinations of IR with other analytical tools:

IR technique can be applied more usefully by combining it with a number of other analytical tools. Some of these useful IR combinations are as follows.

ATR-IR: Attenuated total reflectance technique is combined with IR to analyse solids, powders, pastes and liquids. This technique is very useful in studying the vapour-solid interactions during chemical vapour deposition and to study heterogeneous catalysis.

DRIFTS-IR: DRIFTS is Diffuse Reflectance Infrared Fourier Transform

Spectroscopy. This technique is used in surface chemistry studies of high surface area powders more usefully in heterogeneous catalysis. It is also used for the analysis of polymers. A combination of DRIFTS and near IR is applied to pharmaceutical analysis.

Long Path Gas Cell: this technique involves an increase in the pathlength at a fixed concentration of a gas to get increased IR absorption signals and is used for the analysis of gases (FTIR 2008).

Pharmaceutical applications of IR:

IR did not develop very quickly to be used in the pharmaceutical industry, but still it has gained a good deal of popularity in pharmaceutical industry with respect to research, production and quality control. Some of the most useful applications of IR in pharmaceutical analysis are listed below.

a). Identifications of raw materials and intermediates

b). Analysis of intact dosage form including the analysis of tablets and the analysis of capsules including both hard and soft capsules.

Quantitative analysis applications of IR:

It is based on the determination of one of the functional groups of the compound to be analyzed. The quantity is then calculated using Beer's law.

Draw backs of IR:

IR has the following draw backs

1. Sample cells are made of potassium or sodium chloride therefore the sample thickness may vary due to the following reasons
    a) Due to chemical attack from the solvent
    b) They are easily distorted as they are very soft
2. As the Beer law is used for quantitative analysis using IR, therefore the results obtained may deviate from Beer law due to the following reasons

a) Variation of certain frequencies, shapes and orientations.
b) False measurements produced by the spectrometer of transmitted or absorption bands during some analysis.

Limitations of IR:

IR has some limitations as well. Some of the IR limitations are listed as under

a) It can not be used to find the molecular weight of a substance (except under some certain conditions).
b) Does not provide any information about the position of functional groups in a compound.
c) IR of unknown compounds does not state that if it is a pure compound or a mixture of compounds. Although the IR has the draw backs and limitations as listed above, still it is advantageous in many ways.

2.4.5. Advantages of IR

a) Provides structural information about the structure of molecules
b) Offers a shorter time of analysis (1 to 2 minutes)
c) Minimal or no sample preparation is required prior to analysis.
d) Nutritionally relevant parameters can be predicted.
e) Many parameters can be assessed simultaneously.
f) Minimal operator skills are required.

2.4.6. Disadvantages of IR:

IR has the following disadvantages

a) The purity of a compound is difficult to be determined using a single IR spectrum of an unknown substance
b) Infrared analyses are based on correlations derived from samples with known composition.
c) It is not able to predict all the parameters, most importantly organic compounds present at concentrations below 1g / kg and minerals.
d) IR equipment is quite expensive.

2.5. Thin layer chromatography:

This section explains the theoretical concept of TLC, it's experimental and it's various applications.

2.5.1. Experimental of TLC technique:

The experimental technique of TLC comprises the following main components.

1. **Coating material:** There is a large number of coating materials commercially produced for TLC coating.
2. **Preparation of thin layers:** TLC thin layers are prepared in different ways by using the coating slurry in either way of pouring, spraying, spreading or using pre-coating plates.
3. **Activation of adsorbent:** The adsorbent is activated by drying the plates in air for 30 minutes and then in the oven for another 30 minutes.
4. **Sample application:** It is usually done by using a micro-syringe (for quantitative analysis) and capillary tube for qualitative analysis.
5. **Development tank:** The tank used in paper chromatography is also used in TLC.
6. **Solvent system:** Solvent selection is very important for the TLC analysis is very important as different analysis needs different solvent.
7. **Development method:** Normally ascending technique is used for developing TLC chromatograms.
8. **Detection of components:** The methods of detecting the solutes which are used in paper chromatography are used in TLC as well.
9. **Evaluation of chromatogram:** Evaluation of chromatogram is done either qualitatively or quantitatively.

Applications of TLC:

TLC has been applied very successfully to analyze various organic and inorganic compounds. Its success is mainly due to the properties which the TLC is popular for such as its application to the analysis of most of the chemical compounds, its high speed of separation and high selectivity.

Some of the most common and important applications of TLC are listed as under.

- It is used to check the purity of sample compounds
- It is used as a purification process
- It is a useful tool for examining the reactions
- It is also used to identify compounds
- It is also used in quantitative analysis

To gain the desired results in the analysis mentioned above, it is very important that the reaction is separable and sufficiently nonvolatile to remain on the plate during the development process and is not affected by the developing solvent and adsorbent. Following compounds are analysed by successful application of TLC.

a) It is successfully applied to the analysis of organic acids, alcohols, glycols, alkaloids, amines and amino acids, proteins and peptides.
b) It is applied to antibiotic analysis.
c) It is used for the analysis of inorganic ions.

2.5.3. Advantages of TLC:

Following are some of the advantages which TLC has over the other chromatographic techniques.

1. It is very simple technique
2. It offers a shorter development time for the separation of inorganic adsorbent layers
3. Wide choice of stationary phase
4. Very early recovery of separated components
5. Very easy visualization of separated components
6. Extremely sharp spots are obtained for quantitative analysis
7. It offers a variable thickness of thin layers
8. Uses chemically inert stationary phase

2.5.4. Limitations of TLC:

The main limitation of TLC is that it is used only for small scale preparative works. It is highly desirable that the scale of operation should be increased without simultaneously sacrificing the resolving power. Some workers have obtained high resolution by using column chromatography while others have used film technique. However, a limited success has been achieved in both cases.

Chapter 3:

"CE Method Development and Solvent Selection for the Analysis of Amoxicillin"

Before analysing amoxicillin using capillary electrophoresis, it was necessary to develop a CE method suitable for good analysis and achieving good results e.g. repeatable and accurate. The details of developing a CE method for the analysis of amoxicillin are as follows.

Initial CE conditions: The initial CE conditions used for the analysis of amoxicillin are listed in the Table 3.

Table.3: Initial CE conditions for amoxicillin analysis.

Solvent	50 % HPLC grade water / 50 % methanol
Running buffer (mobile phase)	0.02 M borate phosphate and 1.44% SDS adjusted to a pH 8.60
Column	Agilent technology (40 cm X 50 µm I.D)
injecting pressure	50 mbar
Applied voltage	ranging from 15 kV to 30 kV
Detection wavelength	214nm
Lab temperature	25 Cº
injection type	Hydrodynamic

3. Standard preparation 1:

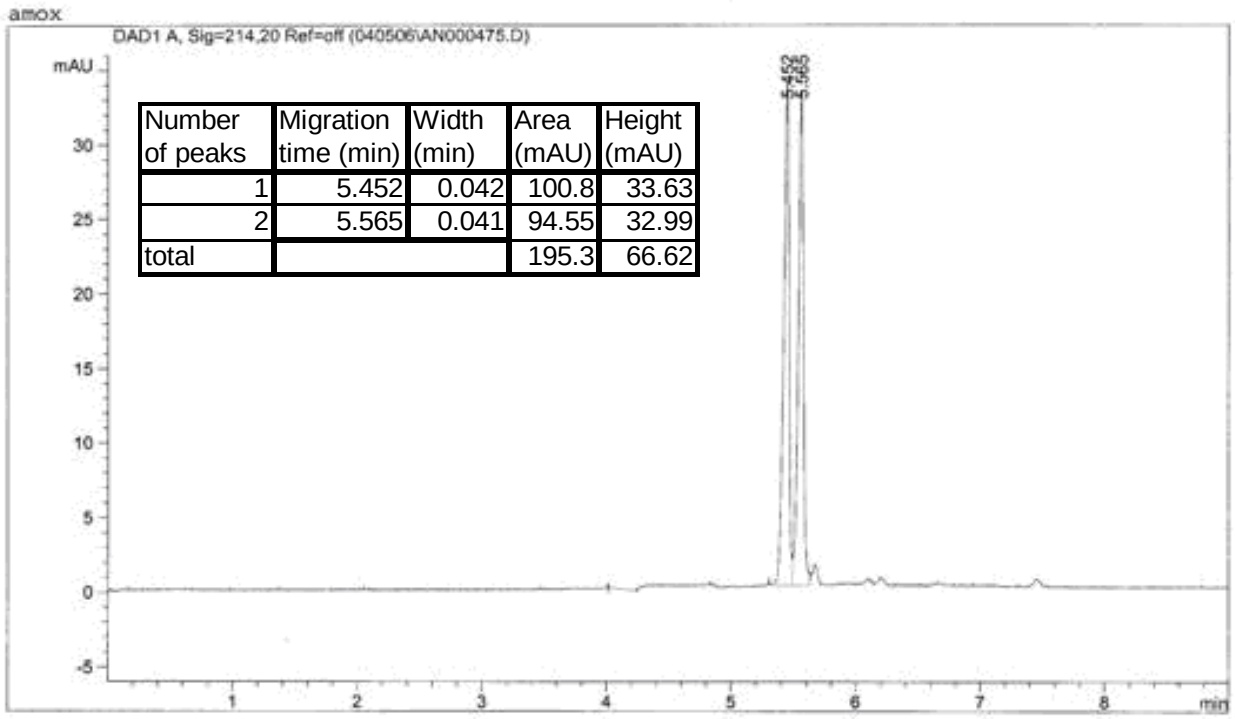
Standard stock solution of a 1000 ppm concentration was prepared by dissolving a weighed amount of standard amoxicillin in a solvent of 50 % HPLC grade water and 50 % HPLC grade methanol. The buffer used was 0.02M borate-phosphate supplemented with 1.44 % SDS having a pH range from 6.0 - 9.0. Finally the buffer was adjusted to a pH 8.60.

3.1. CE procedure 1:

A 100 ppm solution was prepared by diluting the 1000 ppm stock solution. This 100 ppm solution was then transferred to a CE vial through a dropper. The vial was then placed in its proper position in the CE apparatus and injection was performed at an injecting pressure of 50 mbars.

The electropherogram obtained from the first injection, contained two narrow peaks (produced after 5.452 and 5.565 minutes) of equal heights by a difference of three seconds only. The standard was run for three times under the same conditions and the electropherograms obtained were the same as produced from the first injection.

The electropherogram obtained from the first injection are given in the following Figures.



Number of peaks	Migration time (min)	Width (min)	Area (mAU)	Height (mAU)
1	5.452	0.042	100.8	33.63
2	5.565	0.041	94.55	32.99
total			195.3	66.62

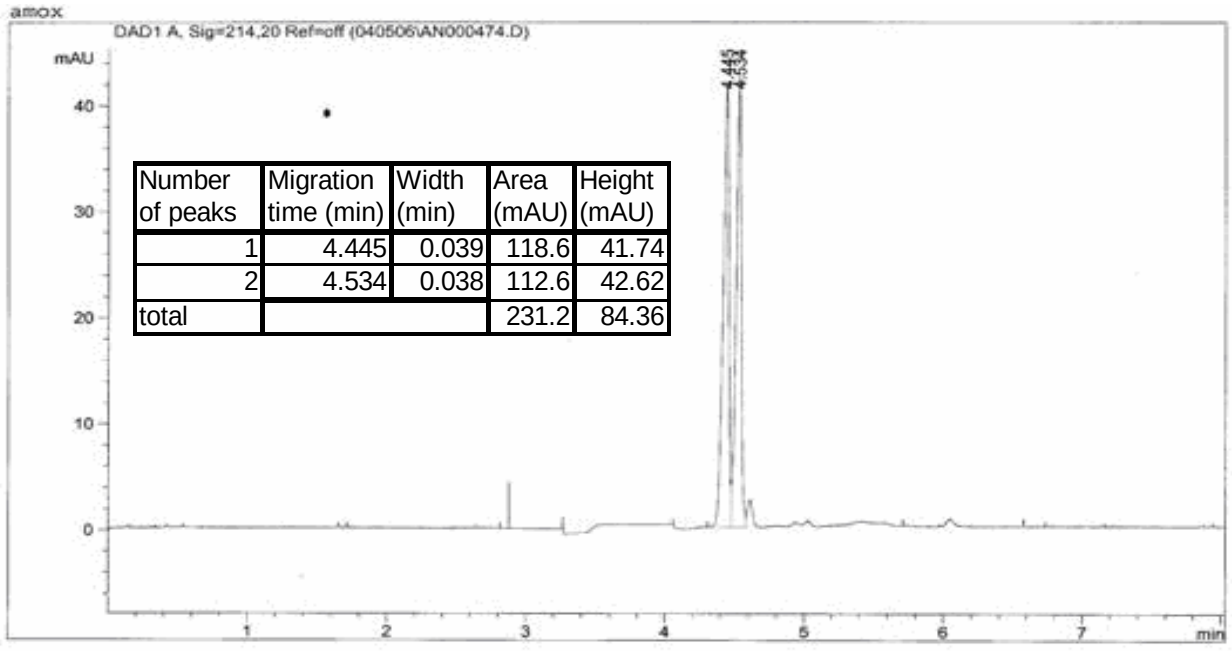
Figure.3. CE electropherogram for pure amoxicillin standard solution of 100 ppm in 50 % methanol / 50 % HPLC grade H_2O at 15 kV.

In order to get a single and well defined peak for amoxicillin standard solution, various parameters were changed one by one and it was observed that the electropherogram had the same two narrow peaks. The parameters which were changed during the analysis are listed below.

- Voltage
- Detection wavelength
- Buffer pH and
- Buffer composition

Any changes in the above parameters had no effects on the peak produced. An electropherogram obtained at 18 kV is given in Figure 3.1.

It is found that a change in voltage produces change in retention time and has no effects on the quality of peak. By increasing the voltage from 15 kV to 18 kV, the migration time decreased from 5.452 and 5.565 minutes to 4.445 and 4.534 minutes.



Number of peaks	Migration time (min)	Width (min)	Area (mAU)	Height (mAU)
1	4.445	0.039	118.6	41.74
2	4.534	0.038	112.6	42.62
total			231.2	84.36

Figure.3.1: Electropherogram for 100 ppm Amoxicillin standard solution at 18 kV when all other experimental conditions were kept the same.

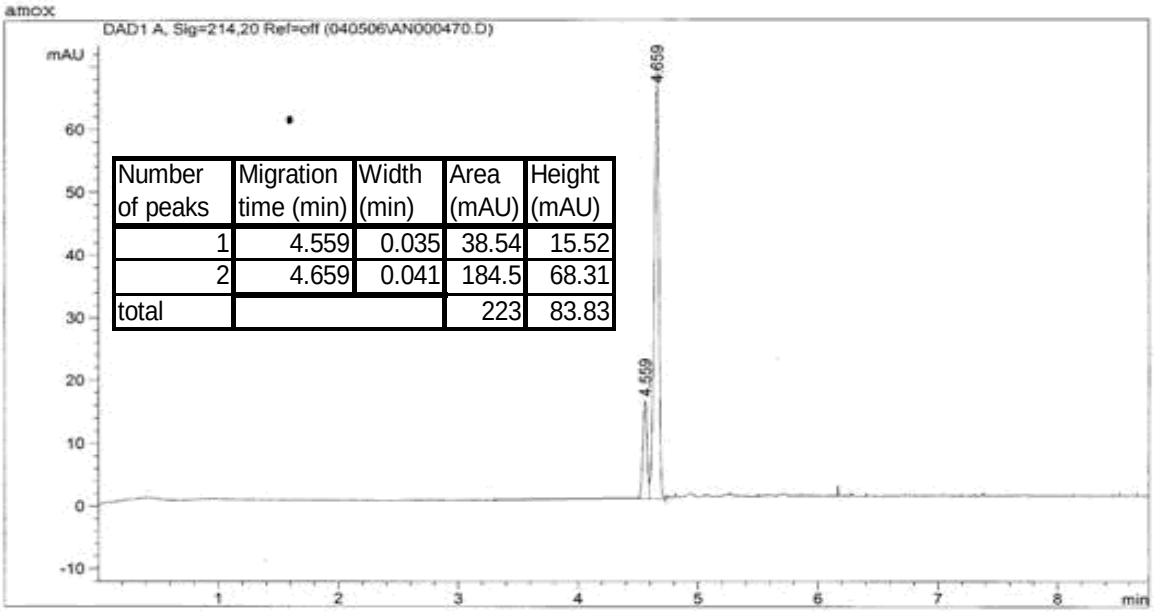
3.1.1 The effects of solvent composition:

After having tried changing different parameters, the solvent composition was slightly changed by the following two ways

1. Increasing methanol by 10 % from 50 to 60 % and decreasing water by 10 % from 50 to 40 %.
2. Decreasing methanol by 10 % from 50 to 40 % and increasing water by 10 % from 50 to 60 %.

The increase in methanol percentage did not affect the peak. However, by decreasing the methanol percentage, it was found that one of the two narrow peaks became smaller.

The percentage of methanol was further reduced such that the solvent composition was 60 % water / 40 % methanol, 80 % water / 20 % methanol. It was found that one of the two narrow peaks was considerably reduced. The electropherogram obtained at a reduced methanol concentration is given in Figure 3.1.1 on the following page. All other experimental conditions were kept the same as for previous CE injection.

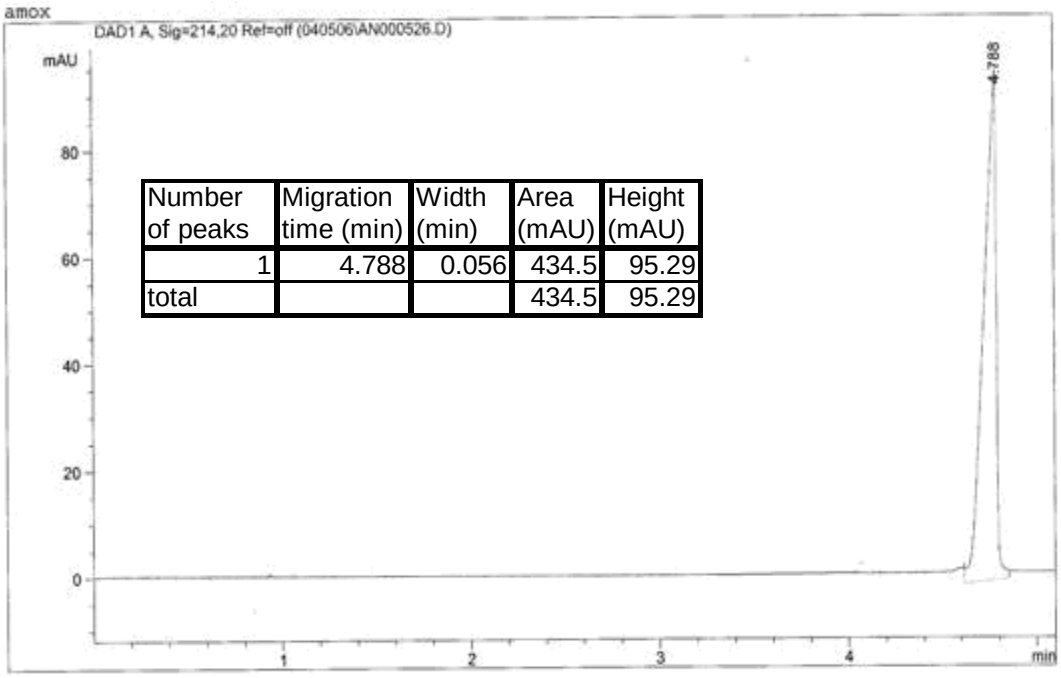


Number of peaks	Migration time (min)	Width (min)	Area (mAU)	Height (mAU)
1	4.559	0.035	38.54	15.52
2	4.659	0.041	184.5	68.31
total			223	83.83

Figure.3.1.1: Electropherogram for 100 ppm Amoxicillin standard solution at the same experimental conditions when methanol concentration was lowered.

Finally when standard solution was prepared in HPLC grade water only without adding any methanol, and was anlysed by CE, a well defined and single peak was produced. This lead to the conclusion that the peak doubling effect was due to the presence of methanol in the solvent composition and proved the water to be a solvent as used in the CE analysis of amoxicillin.

The electropherogram obtained from the analysis of 100 ppm Amoxicillin standard solution at 18 kV contained a single well defined peak when the amoxicillin solution was made with only HPLC grade water. This solution did not have any methanol percentage at all. The electropherogram is shown in the Figure 3.1.2.



Number of peaks	Migration time (min)	Width (min)	Area (mAU)	Height (mAU)
1	4.788	0.056	434.5	95.29
total			434.5	95.29

Figure.3.1.2: electropherogram obtained for 100 ppm Amoxicillin standard solution under the same experimental conditions using only HPLC grade water as a solvent.

3.1.2. Effects of change in voltage on migration time:

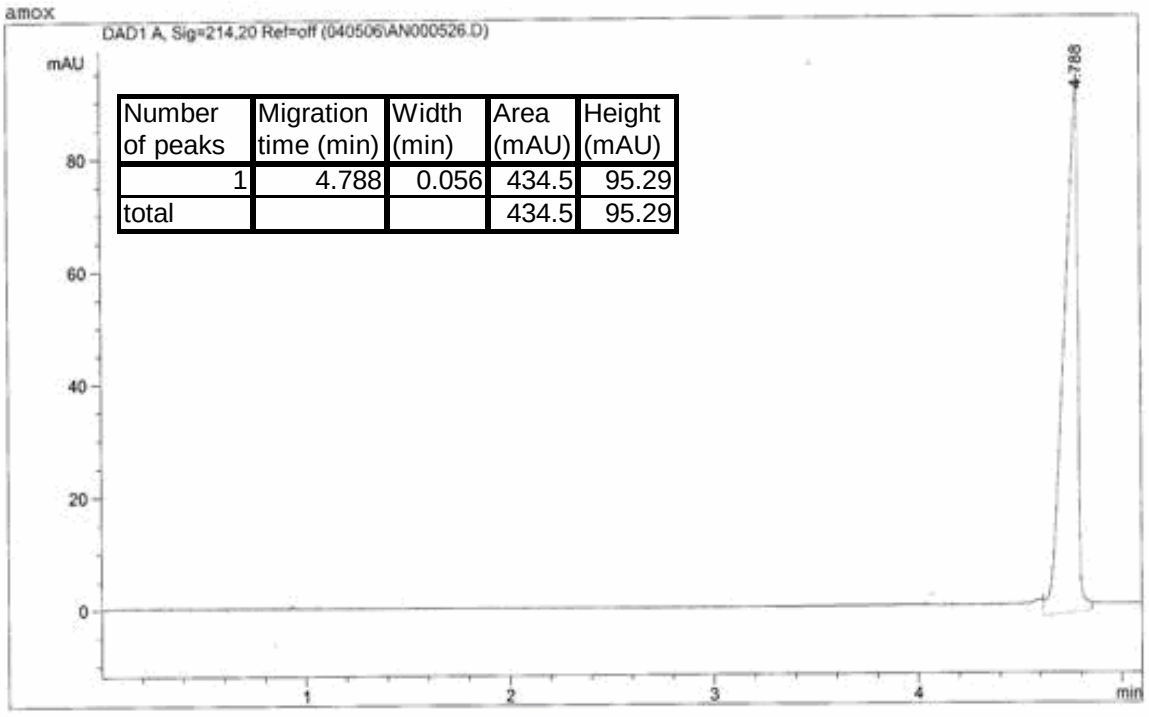
A change in the voltage during the CE analysis of amoxicillin produced a

prominent effect on the migration time. It was found that an increase in the voltage supply decreases the migration time and vice versa. The migration time noted at different voltages is listed in the following Table.

Table.3.1: Effects of voltage on migration time.

Voltage	13 kV	15kV	18kV	30kV
Migration time (min)	7.195	6.390	4.736	2.463

The effects of voltage on migration time can be better understood by viewing the electropherograms for 100 ppm Amoxicillin solution using different values of voltage. These electropherograms are shown in the following Figures.



Number of peaks	Migration time (min)	Width (min)	Area (mAU)	Height (mAU)
1	4.788	0.056	434.5	95.29
total			434.5	95.29

Figure.3.1.3. Electropherogram for 100 ppm amoxicillin solution observed at 18 kV to show the effect of voltage on migration time. The initial migration time recorded is 4.788 minutes.

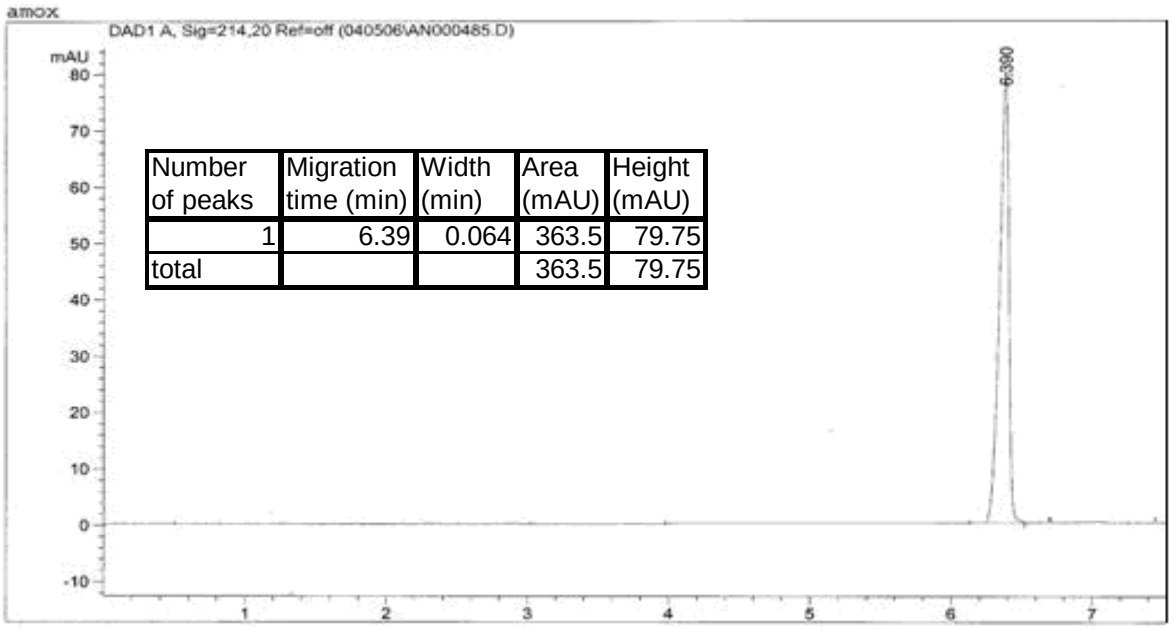


Figure.3.1.4: Electropherogram for 100 ppm amoxicillin solution observed at 15 kV. The migration time increases when the voltage decreases.

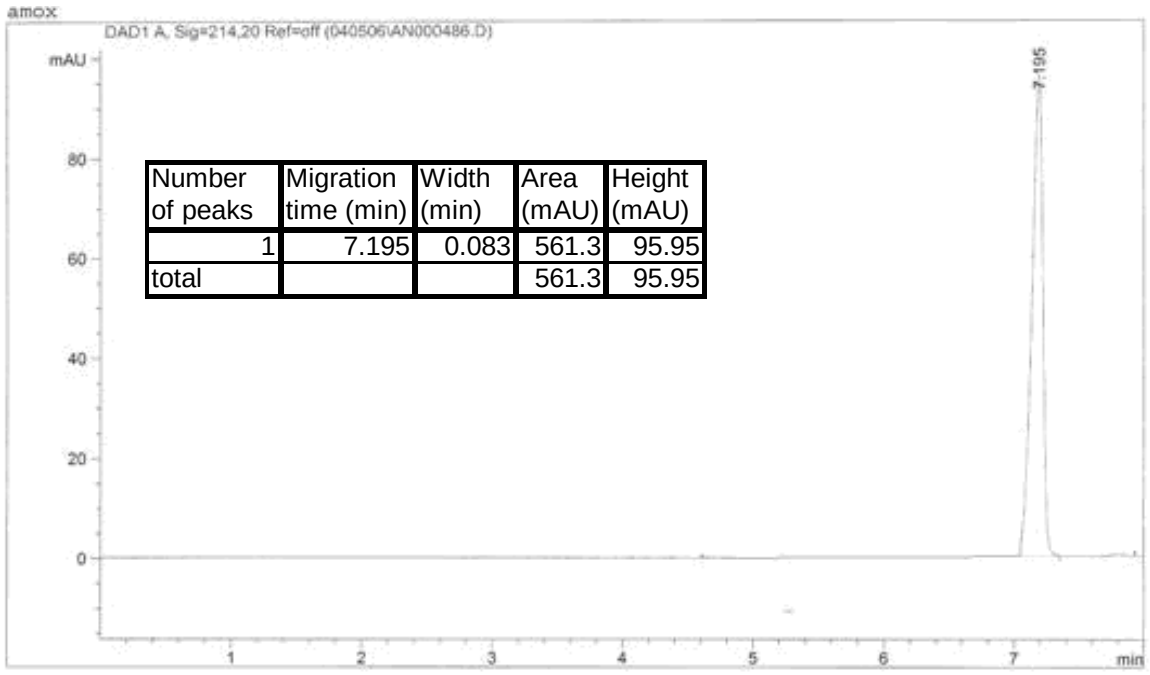


Figure.3.1.5: Electropherogram for 100 ppm amoxicillin solution observed at a voltage of 13 kV. The retention time further increases as the voltage decreases.

It was concluded from the results observed in the electropherograms given above that a decrease in voltage produces an increase in the migration time i.e. decreasing the voltage, an increase in the migration time is observed and vice versa.

3.1.3. Finalising CE Conditions for further Analysis:

After the solvent was selected and having set all the parameters, the CE analysis of amoxicillin was undertaken by using the conditions given in the Table 3.1.1.

Table.3.1.1: CE conditions for the analysis of Amoxicillin.

Solvent	HPLC grade water
Running buffer	0.02 M borate phosphate and 1.44% SDS adjusted to a pH 8.60
Column	Agilent technology (40 cm X 50 µm I.D)
injecting pressure	50 mbar
Applied voltage	ranging from 15 kV to 30 kV
Detection wavelength	214nm
lab temperature	25 Cº
injection type	Hydrodynamic

All the standard amoxicillin solutions of different concentrations and all amoxicillin samples were analysed by following the CE procedure set up as described previously.

Chapter 4: "Experimental Procedures"

This chapter comprises of the designation of all the experimental procedures for the analysis of antibiotic amoxicillin.

The antibiotic amoxicillin was analysed using the following analytical techniques.

- Capillary Electrophoresis
- High Performance Liquid Chromatography
- Infrared Spectroscopy

4.1. Capillary Electrophoresis of Amoxicillin:

Capillary electrophoresis experiments were carried out on Agilent CE system, equipped with a power supply of 30kV, and a UV spectrophotometer detector connected to a data collection system. The system was able to perform both hydrodynamic and voltage injection.

Reagents and Standards:

Pure Amoxicillin tri-hydrate (of 97% purity) was obtained from Aldrich. Sodium phosphate, sodium tetra borate, sodium hydroxide and phosphoric acid were of reagent grade. Sodium Dodecyl sulfate (SDS) was provided by Aldrich. Water used to prepare standard and sample solutions, and the running buffers were also obtained from Aldrich. Samples analysed were obtained from different parts of subcontinent and the Middle East. All the samples were weighed and then the same samples were analysed on HPLC, CE and IR. These samples are listed in the Table 4 on the following page.

Table.4: Amoxicillin samples.

Amoxicillin Sample	**Pharmaceutical company**
Maxil 500 mg	Macter International, Karachi, Pakistan
Werrimox 500 mg	Werrick Pharmaceuticals, Islamabad, Pakistan
Amoxicillin 500 mg	Pliva Pakistan, Baluchistan, Pakistan
Effimox 500 mg	Wilson's Pharmaceuticals, Islamabad, Pakistan
Namoxil 500 mg	Nawan Laboratories, Karachi, Pakistan
Amoxascot 500 mg	Scotmann Pharmaceuticals, Islamabad, Pakistan
Medimox 500 mg	Medicraft Pharmaceuticals, Hayatabad, Peshawar Pakistan
Labmox 500 mg	Laborate Pharmaceuticals, India
HMC 250 mg	H-Tech International enterprises, Hayden Beijing, China
Acamoxil 250 mg	ACAI Pharmaceuticals, Iraq
Glomox 500 mg	Glow expert trading, Mumbai, India

Standard preparations:

Standard stock solution of a 1000 ppm (1000 mg / l) concentration was prepared by dissolving a weighed amount of standard amoxicillin in HPLC grade water. The buffer used was 0.02 M borate-phosphate having a pH range from 6.0-9.0, supplemented with 1.44 % SDS. Finally the buffer was adjusted to a pH 8.60.

CE conditions:

The CE analysis of Amoxicillin was undertaken by using the following conditions as mentioned in the Table 4.1 on the following page.

Table.4.1: CE conditions for the analysis of Amoxicillin.

Solvent	HPLC grade water
Running buffer	0.02 M borate phosphate and 1.44 % SDS adjusted to a pH 8.60
Column	Agilent technology (40 cm X 50 µm I.D)
injecting pressure	50 mbar
Applied voltage	ranging from 15 kV to 30 kV
Detection wavelength	214nm
lab temperature	25 C^{o}
injection type	Hydrodynamic

CE procedure:

A 100 ppm solution was prepared by diluting the 1000 ppm stock solution. This 100 ppm solution was then transferred to a CE vial through a dropper. The vial was then placed in its proper position in the CE apparatus and injection was performed at an injecting pressure of 50 mbars and detection wave length of 214 nm.

Determination of migration time (M_t) for standard solutions:

Once a suitable CE method was developed for the analysis of Amoxicillin, CE injection was performed for all the standard solutions (300 ppm, 200 ppm, 150 ppm, 100 ppm and 50 ppm) one by one and the migration times for all the standards were recorded and tabulated as under in Table 4.1.1.

Table.4.1.1: Migration times recorded for various Amoxicillin standards solutions.

amoxicillin standards	50 ppm	100 ppm	150 ppm	200 ppm	300 ppm
migration time (min)	4.759	4.673	4.768	4.775	4.681

The effect of voltage on migration time was previously discussed that voltage inversely affects migration time. The slight variations in migration time noted for different concentrations of amoxicillin standard solutions is due to the slight

variation in room temperature which also can affect the voltage supply and varying migration time.

Migration time variation was also observed when the buffer efficiency decreases because of repeated CE analysis. The migration time did not show any further variation when the temperature was kept constant by air conditioning the CE lab and by changing the buffer vials frequently.

Sample preparation:

Eleven samples from different countries were collected and then three capsules form each sample were analysed. All the samples were in capsule form and their contents were directly analysed without any further purification. Masses of the sample capsules were recorded using an analytical balance. Then 0.10 g of each capsule was taken in three separate 50 cm^3 volumetric flasks and the sample solutions were prepared to these volumes using the HPLC grade water as a solvent.

The sample solutions were then placed in an ultrasonic bath for achieving a complete dissolution. After getting dissolved the samples, the sample solutions were then centrifuged to separate any solid sample particles left in the solutions. The reason for centrifugation was to protect the narrow capillary from being blocked by small solid particles in the sample solution.

Sample injection and migration times of samples:

Once all the sample solutions were ready, they were analysed by CE and their migration times were noted. Standard solutions of pure amoxicillin of different concentrations (500 ppm, 1000 ppm, 1500 ppm, 2000 ppm and 2500 ppm) were always analysed before analysing each sample solution. The migration times noted for all the samples are listed in the Table 4.1.2 on the following page.

Table.4.1.2: Migration times recorded for Amoxicillin samples.

Amoxicillin samples	Migration time in minutes
Maxil 500 mg	4.696 min
Werrimox 500 mg	4.708 min
Amoxicillin 500 mg	4.819 min
Effimox 500 mg	4.693 min
Namoxil 500 mg	4.957 min
Amoxascot 500 mg	4.840 min
Medimox 500 mg	4.761 min
Labmox 500 mg	4.701 min
HMC 250 mg	4.652 min
Acamoxil 250 mg	4.671 min
Glomox 500 mg	4.628 min

The migration times noted for all the Amoxicillin samples are almost the same. The minor differences found between the migration times are probably due to slight variation of temperature of laboratory during analysis. Migration time variation was also observed when the buffer efficiency decreases because of repeated CE analysis. The migration time did not show any further variation when the temperature was kept constant by air conditioning the CE lab and by changing the buffer vials more frequently.

4.2. HPLC Analysis of Amoxicillin:

A review of literature revealed that several High Performance Liquid Chromatography methods have been reported for the individual identification and quantification of amoxicillin in the sample or determination of amoxicillin in a mixture of other compounds.

Here, the method used is an isocratic reversed phase HPLC method for the determination of amoxicillin in the sample capsules using Kontron 320 HPLC system connected to Shimadzu C-RSA integrator and a UV detector of variable wavelength.

Chromatographic system:

HPLC chromatographic system consisted of a pump equipped with a 50 μl loop, ultraviolet variable wavelength detector. All the chromatographic separations were achieved on an Ultra C18 column (150 mm x 4.6mm I.D) as a stationary phase.

Reagents and chemicals:

Standard amoxicillin was purchased from Aldrich (of 97 % purity) and was used without any further purification. HPLC grade water and HPLC grade methanol was purchased from sigma and used to prepare all the standards.

Chromatographic conditions:

All the chromatographic conditions for the analysis of Amoxicillin are given in the Table 4.1.3 on the following page.

Table.4.1.3: Chromatographic conditions for HPLC analysis of Amoxicillin.

solvent / mobile phase	60 % HPLC grade water / 40 % methanol
Column	C - 18
Elution	Isocratic reverse phase
Flow rate	1 ml / min
Wavelength	230 nm
Temperature	Room temperature (20 Cº to 25 Cº)

Standard preparations:

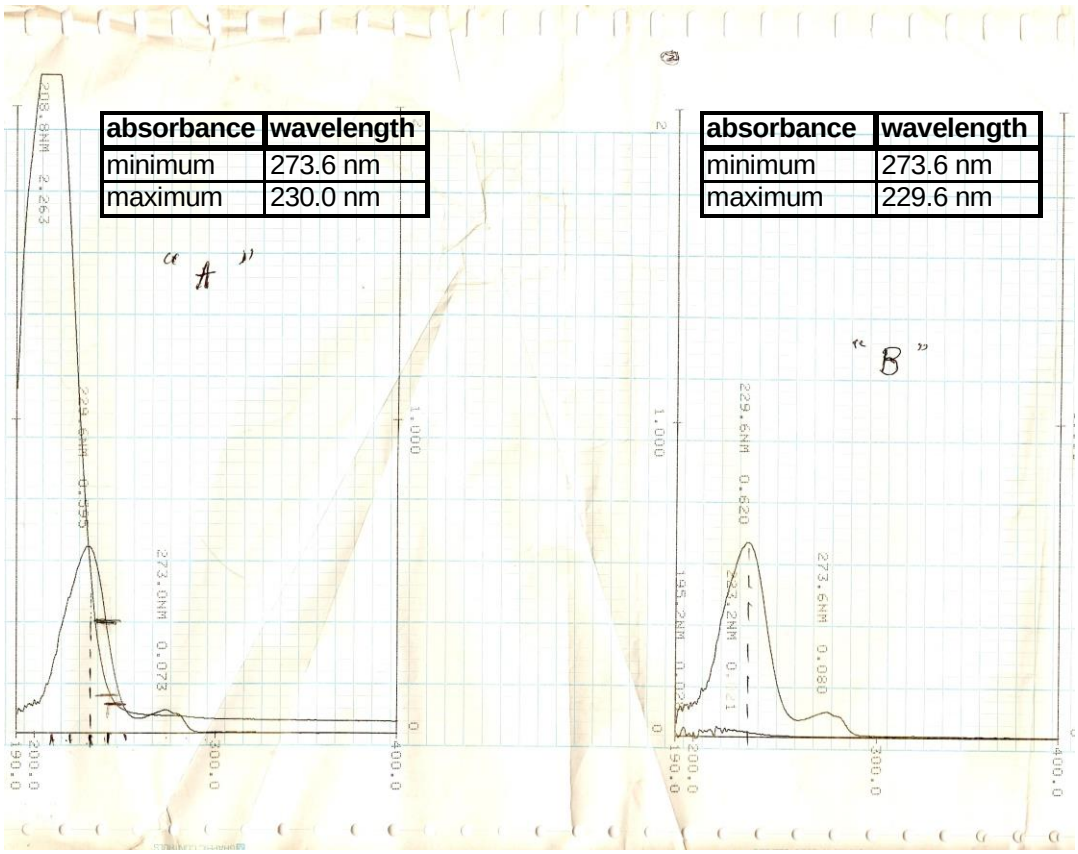
A standard Amoxicillin solution (stock solution) of 1000 ppm (1000 mg / l) was prepared by dissolving a weighed amount of pure Amoxicillin in the solvent having same composition as the mobile phase mentioned above.

Standard solutions of Amoxicillin having concentrations of 30 ppm, 20 ppm, 15ppm, 10 ppm and 5 ppm were prepared by diluting the 1000 ppm solution (stock solution) using the same solvent.

Selection of detection wavelength for Amoxicillin:

In order to select the wavelength to be used by the UV detector of HPLC, a UV spectrum of pure Amoxicillin (standard solution of 100 ppm) was obtained using a UV spectrometer. The UV spectrometer was set at a wave length ranging from 190 nm to 400 nm. The optimum wave length found for pure Amoxicillin was 230 nm. The UV spectrum obtained is given in the Figure 4 on the following page.

Figure.4: UV spectrum shown by amoxicillin standard solution of 100 ppm at a wavelength ranging from 190 nm to 400 nm.



Where

A = amoxicillin and mobile phase **B** = mobile phase only

Determination of retention time (R_t) for standard solutions:

Once the detection wavelength was selected, all the Amoxicillin standard solutions (30 ppm, 20 ppm, 15 ppm, 10 ppm and 5 ppm) were injected on to the HPLC system one by one and the retention times for all the standard solutions were recorded and are given in the Table 4.1.4.

Table.4.1.4: Retention times recorded for various Amoxicillin standards solutions (variation found is probably due to the variation in laboratory conditions).

amoxicillin standards	5 ppm	10 ppm	15 ppm	20 ppm	30 ppm
retention time (min)	4.99	4.84	4.98	5.11	5.01

Table 4.1.4 shows that the retention time noted for different concentrations of

amoxicillin standard solutions is not same and shows a slight variation. The retention time depends on temperature, contamination build up in the column and flow rate. As the flow rate remains the same throughout the analysis therefore, this minor variation is probably due to the change in room temperature and contamination which builds up due to repeated analysis without flushing the capillary out.

If the HPLC laboratory temperature is kept constant and capillary is flushed with mobile phase regularly between each two analysis, the retention time noted for different concentrations of standard solutions of the same compound should remain the same.

Sample preparation:

Eleven samples from different countries were collected and then three capsules from each sample were analysed. All the samples were in capsule form and their contents were directly analysed without any further purification. Masses of the sample capsules were recorded using an analytical balance. Then 0.10 g of each capsule was taken in three separate 50 cm^3 volumetric flasks and the sample solutions were prepared to these volumes using the mobile phase as a solvent. In order to dissolve the sample completely and get clear sample solutions, the sample solutions were sonicated until clear solutions were prepared.

Sample injection and retention time:

After preparing completely dissolved sample solutions, these solutions were injected onto the HPLC system one by one and retention times were then noted for each sample solution. Standard solutions of pure amoxicillin having different concentrations (200 ppm, 400 ppm, 600 ppm, 800 ppm and 1000 ppm) were also injected as a reference before injecting the samples every time.

The retention times recorded for amoxicillin samples are listed in the Table 4.1.5 on the following page.

Table.4.1.5: Retention times recorded for Amoxicillin samples.

Amoxicillin samples	Retention time in minutes
Maxil 500 mg	4.203 minutes
Werrimox 500 mg	4.215 minutes
Amoxicillin 500 mg	4.243 minutes
Effimox 500 mg	4.238 minutes
Namoxil 500 mg	4.23 minutes
Amoxascot 500 mg	4.22 minutes
Medimox 500 mg	4.198 minutes
Labmox 500 mg	4.195 minutes
HMC 250 mg	4.361 minutes
Glomox 500 mg	4.712 minutes
Acamoxil 250 mg	4.238 minutes

Table 4.1.5 shows that there is a slight variation in retention times noted for different brands of amoxicillin samples. Some of the samples eluted slightly faster than the others. The reason for this change in retention time was the changing temperature of the HPLC lab. When the samples were analysed at constant laboratory temperature, the retention time noted for all the samples was same (4.22 minutes to 4.23 minutes).

Second cause for this slight change in retention time was the contamination build up by repeated sample analysis without flushing the column after each sample injection. When the column was flushed with solvent in between each two analysis, the retention time noted for all the amoxicillin samples was the same producing no variation.

4.3. The Infrared Spectroscopy of Amoxicillin:

The infrared spectroscopy of amoxicillin was carried out using the IR spectrophotometer (Nicolet 210) for recording spectra consisting of a light source, monochromator and a detector.

Standards and Reagents:

Potassium bromide, Standard amoxicillin tri-hydrate of 97% purity was purchased from Aldrich. The samples analysed have already been listed in the beginning of this chapter and were obtained from Middle East and Subcontinent.

Preparation of samples:

The method used for the preparation of samples was the disc method. Before analysing the samples of various brands of amoxicillin, pure amoxicillin was analysed by IR. The disc formation for pure amoxicillin is as follows.

1 mg of pure amoxicillin was mixed with 100 mg of potassium bromide. The mixture was ground and was pressed under a high pressure of about 800 MPa until a small round transparent disc was formed. The disc was then placed within the appropriate holder FT – IR spectrometer and scanned between 4000 cm^{-1} and 400 cm^{-1}. The disc method described above for the IR analysis of pure amoxicillin was used for the analysis of different brands of amoxicillin samples.

The spectrum obtained can be explained with the help of assigning absorbance to their respective functional groups in amoxicillin as each functional group shows a characteristic absorbance at a particular wave length as shown in the Figure 4.1 on the following page.

Figure.4.1: IR spectrum for pure Amoxicillin.

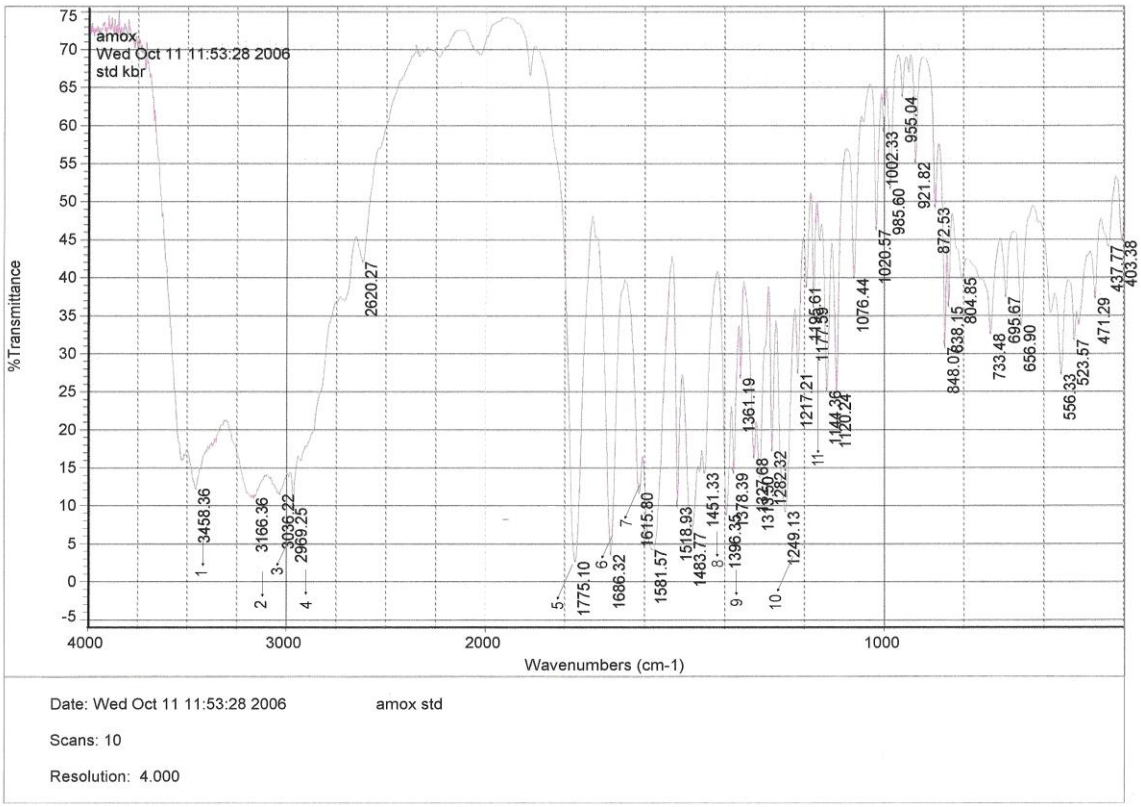


Figure.4.1.1: Structure of Amoxicillin.

The IR peaks observed for pure Amoxicillin are marked with arrows and are assigned to their respective functional groups. These are listed in the Table 4.1.7.

Table.4.1.7: List of IR peaks absorbance for pure Amoxicillin as assigned to their respective functional groups.

Peak. No	Functional Group	Absorbance
1	Cyclic amide	3457.98 cm^{-1}
2	═C─H in aromatic ring	3166.91 cm^{-1}
3	─CH_3	3036.89 cm^{-1}
4	─CH_3	2969.12 cm^{-1}
5	─C═O stretching in cyclic amide	1775.03 cm^{-1}
6	─C═O stretching in normal amine	1686.41 cm^{-1}
7	N─H bending in normal amine	1615.91 cm^{-1}
8	Skeletal stretch in aromatic ring	1451.30 cm^{-1}
9	─CH_3 of ─CH3─CO by the ring	1396.37 cm^{-1}
10	C─O─C in ─CO_2H	1248.96 cm^{-1}
11	─CH_3 directly attached to the ring	1177.59 cm^{-1}

The bands in 800--600 cm^{-1} appear due to the bending of the ─C─H bond. The bands in the region from 1600—1500 cm^{-1} appear due to C═C bond of the aromatic ring. Whereas, the rest of the spectrum can be regarded as a “fingerprint” of the entire amoxicillin structure.

All the samples were analysed following the same procedure as mentioned above. The spectrum for each of the sample amoxicillin was recorded and two characteristic strong peaks were observed at 1775 cm^{-1} and 1686 cm^{-1} confirming the presence of ─C═O in cyclic amide structure and ─C═O in normal amine group respectively.

Chapter 5: "Results and Discussions"

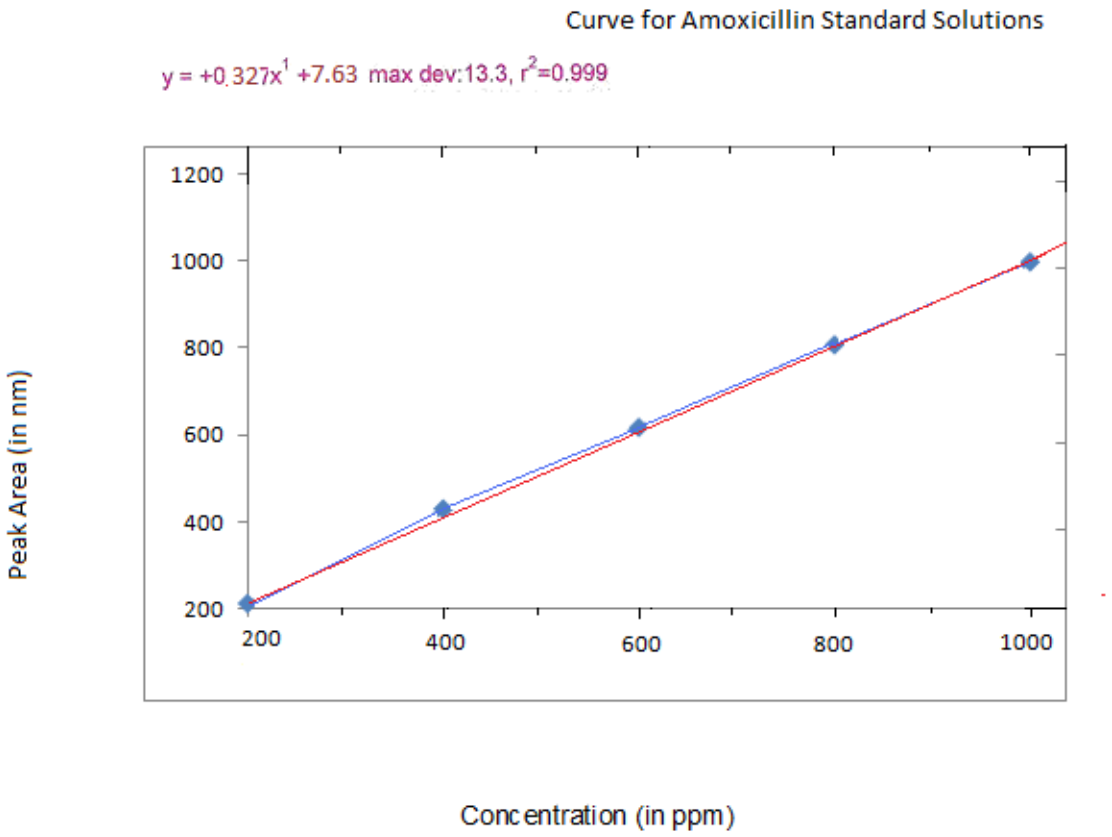
This chapter includes the results produced from the analysis of different brands of antibiotic Amoxicillin. The results for each of the samples will be discussed in this chapter. A list of all the samples is given on the following Table. The HPLC, CE and IR results are discussed together for each sample. The results are listed according to the sample number given in the list in the form of Table 4.

Table.4: List of amoxicillin samples.

Sample number	**Amoxicillin Sample**	**Pharmaceutical company**
1	Maxil 500 mg	Macter International, Karachi, Pakistan
2	Werrimox 500 mg	Werrick Pharmaceuticals, Islamabad, Pakistan
3	Amoxicillin 500 mg	Pliva Pakistan, Baluchistan, Pakistan
4	Effimox 500 mg	Wilson's Pharmaceuticals, Islamabad, Pakistan
5	Namoxil 500 mg	Nawan Laboratories, Karachi, Pakistan
6	Amoxascot 500 mg	Scotmann Pharmaceuticals, Islamabad, Pakistan
7	Medimox 500 mg	Medicraft Pharmaceuticals, Hayatabad, Peshawar, Pakistan
8	Labmox 500 mg	Laborate Pharmaceuticals, India
9	HMC 250 mg	H-Tech International enterprises, Hayden, Beijing, China
10	Acamoxil 250 mg	ACAI Pharmaceuticals, Iraq
11	Glomox 500 mg	Glow expert trading, Mumbai, India

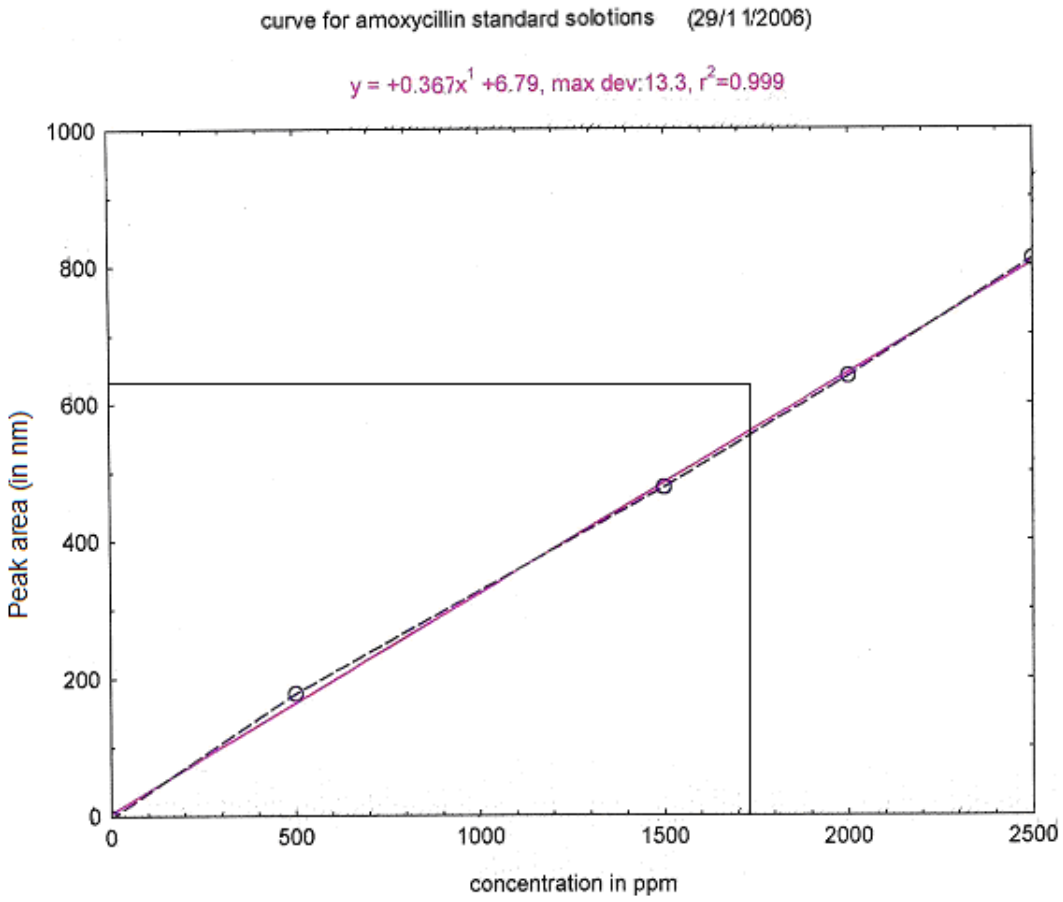
The amount of amoxicillin in all samples was calculated for both CE and HPLC methods after calibration curves were obtained for different amoxicillin standard solutions with the analysis of every single sample. The calibration curves (drawn for calculating the amount of amoxicillin in sample 1a after HPLC analysis) for various concentrations of amoxicillin standard solutions are given in the Figure 5.1a.

Figure.5: Calibration Curves for amoxicillin standard solutions of different concentrations (200, 400, 600, 800 and 1000 ppm) after HPLC analysis.



The calibration curves (drawn for calculating the amount of amoxicillin in sample 1a after CE analysis) for various concentrations of amoxicillin standard solutions are given in the Figure 5 on the following page.

Figure.5.1a: Calibration Curves for amoxicillin standard solutions of different concentrations (500, 1000, 1500, 2000 and 2500 ppm) after CE analysis.



Calibration curve obtained for different standard solutions of amoxicillin shows a linear rise from lower to higher concentrations. Calibration curves were obtained for all the samples to find the amount of amoxicillin in the sample capsules.

The amount of amoxicillin in each of the sample capsule (after CE analysis) was calculated using the following equation

$$y = b\,x + a \qquad (1)$$

Where “y” is the peak area, “x” is the concentration, “b” is the slope and “a” being the intercept on the y-axis.

Now for sample 1a, the amount of amoxicillin after CE analysis was calculated using equation 1.

From the calibration curve given in the Figure 5

$y = 0.367 x + 6.79$ (where 0.367 = b and 6.79 = a)

But $y = 635.132$ (area of the peak produced by sample 1a during CE)

Putting the values in equation 1 we get $635.132 = 0.367 x + 6.79$

Or $x = 635.132 - 6.79 / 0.367$

$x = 1723.8149$

As x = no of µg of amoxicillin in 1 ml of sample solution. Therefore

No of µg of amoxicillin in 1 ml of sample solution = 1723.8149 µg

No of µg of amoxicillin in 50 ml of sample solution = 50 x 1723.8149

$x = 86190.745$ µg

Value of x in mg = 86.1907 mg

Value of x in gm = 0.08619 gm

No of gm of amoxicillin in 0.10 gm of sample = 0.08619 gm

No of gm of amoxicillin in 0.630 gm of sample = 0.630 x 0.08619 / 0.10

= 0.542997 gm

= 0.543 gm

= 543 mg

The amount of amoxicillin for all the other sample capsules from different brands of amoxicillin was calculated using the method as described above for sample capsule 1a. Same method for the calculation of the amount of amoxicillin in the sample capsules was used for all the different brands of amoxicillin analysed by HPLC.

Sample.1. Maxil

CE results:

Three capsules from sample Maxil were analysed and were designated as sample 1a, 1b and 1c.

Table.5.1. CE results produced by Maxil sample capsules.

	Total weight of sample (mg)	Amoxicillin per capsule (as stated) (mg)	Migration Time (min)	Amoxicillin Per capsule (as sampled) (mg)	Number Of Repeats
1a	630	500	4.69	543	3
1b	630	500	4.72	531.3	3
1c	619	500	4.7	514.79	3
Average	**627.7**	**500**	**4.70**	**529.69**	**3.00**
St Dev	**7.8**	**0.0**	**0.02**	**11.57**	**0.00**

Each individual sample capsule was analysed three times repeatedly under the same experimental conditions and the results recorded every time for each individual capsule were the same and the standard deviation calculated for the migration time of each sample was almost equal to zero. The value of standard deviation suggests that repeatability exists within the sample capsules.

Table 5.1 shows that sample Maxil 1a and 1b contain amoxicillin in excess amount compared to the claimed quantity of 500 mg amoxicillin per sample capsule, similarly Maxil sample capsule 1c also contains excess amoxicillin as compared to the claimed amount of 500 mg of amoxicillin per sample capsule.

It is also found that the calculated amounts of amoxicillin per sample capsule are not the same in all the sample capsules analysed. Therefore, a variation in content uniformity within the brand is observed.

The average amount of amoxicillin per sample capsule comes to 529.69 mg

which is still a higher value as compared to the claimed amount of amoxicillin per sample capsule. Despite of slightly higher calculated values of amoxicillin per sample capsule compared to the stated amount, these samples meet the international standards of quality control and are therefore of acceptable standard.

HPLC results:

HPLC results obtained from the analysis of capsules 1a, 1b and1c from sample1 (Maxil) are listed in Table 4.1a.

Table.5.1a: Results obtained from the HPLC analysis of sample Maxil.

	Total weight of sample (mg)	Amoxicillin per capsule (as stated) (mg)	Retention Time (min)	Amoxicillin Per capsule (as sampled) (mg)	Number Of Repeats
1a	630	500	4.73	544.6	3
1b	630	500	4.77	529.45	3
1c	619	500	4.72	517.66	3
Average	**627.66**	**500.0**	**4.74**	**530.57**	**3.00**
St Dev	**6.34**	**0.0**	**0.03**	**± 11.02**	**0.00**

All the three sample capsules were analysed three times repeatedly under the same experimental conditions and the results recorded every time for each individual capsule were the same. This suggested the individual capsules highly repeatable.

Table 5.1a indicates higher values of amoxicillin for all the three Maxil sample capsules as compared to the claimed amount of 500 mg amoxicillin per sample capsule. The average value comes to be 530.57 mg of amoxicillin per capsule of Maxil sample.

It is also found that the calculated amounts of amoxicillin per sample capsule are

not the same in all the sample capsules analysed. Therefore, a variation in content uniformity within the brand is observed. The results suggest that all the sample capsules contained the amount of amoxicillin within the acceptable limits of international standards of quality control and therefore of good standard.

A comparison of CE and HPLC results for sample 1 is given in Figure 5.

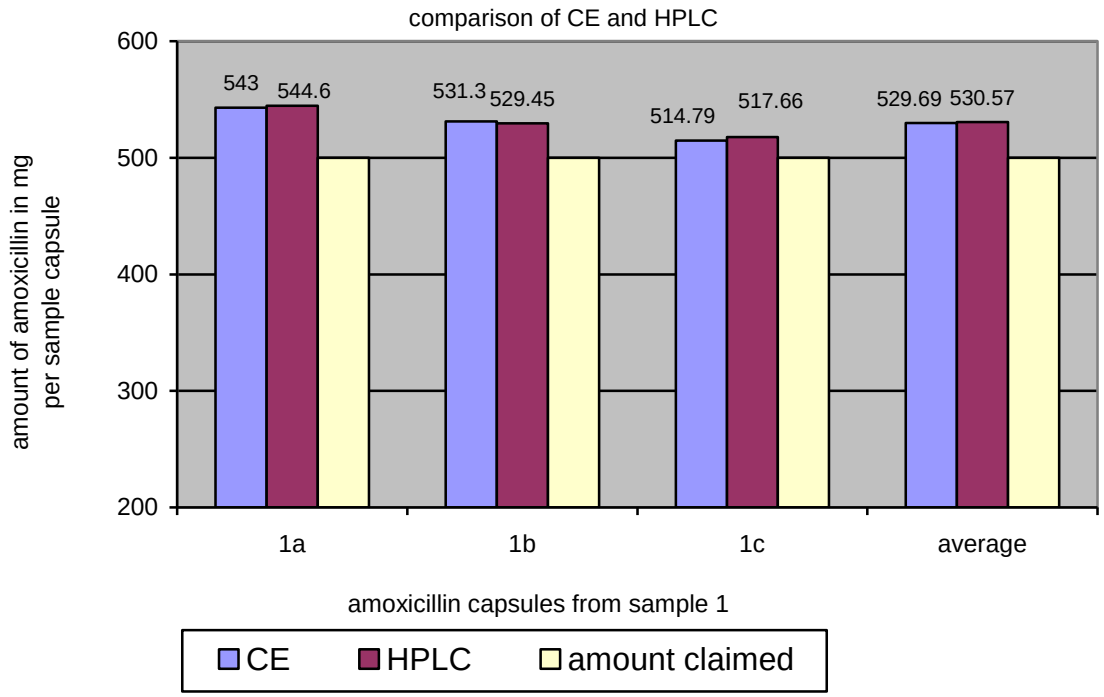


Figure.5.1a: Bar chart showing a comparison of CE and HPLC results for sample 1.

Figure 5.1 shows slightly higher values of amoxicillin calculated per sample capsule by CE method compared to HPLC. The reason for the difference between the HPLC and CE results has not been investigated due to time limits for the project.

IR results:

For further conformation of contents, IR analysis of sample 1 was undertaken

and the spectrum obtained was compared with the IR spectrum of pure amoxicillin. The various peaks observed are listed in Table 5.1b.

Table.5.1b: comparison between strong IR peaks of amoxicillin sample Maxil and pure amoxicillin.

S.No		λ Absorb.(max) $^{-1}$	λ Absorb.(max) $^{-2}$
1	Pure Amoxicillin	1686.32 cm^{-1}	1775.10 cm^{-1}
2	Sample 1a	1686.34 cm^{-1}	1775.21 cm^{-1}
3	Sample 1b	1686.35 cm^{-1}	1775.01 cm^{-1}
4	Sample 1c	1686.37 cm^{-1}	1775.09 cm^{-1}

The characteristic strong peaks observed at 1775.10 cm^{-1} and 1686.32 cm^{-1} were recorded in the IR spectrum of pure amoxicillin and amoxicillin sample Maxil confirming the presence of ─C═O in cyclic amide structure and ─C═O in normal amine group respectively.

The rest of the medium and weak peaks observed in IR spectrum of sample 1 were also compared to those observed in IR spectrum of pure amoxicillin and were found the same.

Summary: The retention and migration time recorded (from HPLC and CE respectively) for all the capsules from sample 1 agree the retention and migration time recorded for pure amoxicillin and also the IR spectra produced by capsules from sample 1 strongly correspond to the IR spectrum of pure amoxicillin. This confirms the presence of amoxicillin in sample 1.

The HPLC and CE analysis show that sample 1 contained relevant amount of amoxicillin more or less the exact amount stated by the manufacturer which meets the international standards of quality control and thus did not show any counterfeiting.

Sample.2. Werrimox

CE results:

Three capsules from sample Werrimox were analysed and were designated as sample 2a, 2b and 2c.

Table.5.2: CE results produced by Werrimox sample capsules.

	Total weight of sample (mg)	Amoxicillin per capsule (as stated) (mg)	Migration Time (min)	amoxicillin per capsule (as sampled) (mg)	Number Of Repeats
2a	598	500	4.7	513.45	3
2b	657	500	4.71	521.6	3
2c	579	500	4.68	518.26	3
Average	**611.3**	**500.0**	**4.70**	**518.6**	**3.00**
St Dev	**40.7**	**0.0**	**0.02**	**5.3**	**0.00**

Each individual sample capsule was analysed three times and the results produced by each individual sample were the same. Standard deviation calculated for migration time noted for each individual sample capsule was almost equal to zero suggesting that repeatability exists within the sample capsules.

As the calculated amount of amoxicillin in the sample capsules of the same brand is not equal and shows variation between the sample capsules, hence a variation in content uniformity is observed.

Table 5.2 indicates higher values of amoxicillin for all the three Werrimox sample capsules as compared to the claimed amount of 500 mg amoxicillin per sample capsule. The average value comes to be 518.57 mg of amoxicillin per capsule of Werrimox sample. The results suggest that all the sample capsules contained the

amount of amoxicillin within the acceptable limits of international standards of quality control and therefore of good standard.

HPLC results:

HPLC results obtained from the analysis of capsules 2a, 2b and 2c from sample 2 (Werrimox) are listed in Table 4.2a.

Table.5.2a: HPLC results for sample Werrimox.

	Total weight of sample (mg)	Amoxicillin per capsule (as stated) (mg)	Retention Time (min)	Amoxicillin per capsule (as sampled) (mg)	Number Of Repeats
2a	611	500	4.78	523.72	3
2b	587	500	4.73	514.35	3
2c	593	500	4.73	514.21	3
Average	**597.0**	**500.0**	**4.75**	**517.4**	**3.00**
St Dev	**12.5**	**0.0**	**0.03**	**5.5**	**0.00**

All the three sample capsules were analysed three times repeatedly using HPLC under the same experimental conditions and the results recorded every time for each individual capsule were the same. This proved the individual capsules highly repeatable.

Table 5.2a indicates higher values of amoxicillin for all the three Werrimox sample capsules as compared to the claimed amount of 500 mg amoxicillin per sample capsule. The average value comes to be 517.42 mg of amoxicillin per capsule of Werrimox sample. The average calculated amount of amoxicillin per sample capsule is a higher value indicating the excess usage of raw material in the manufacturing process of amoxicillin.

Furthermore, sample 2a has higher value of amoxicillin as compared sample 2b and 2c therefore there is a variation in content uniformity within the same brand

of amoxicillin sample. The results produced by all the three sample capsules meet the international standards of quality control and thus were found of good standard.

A comparison of HPLC and CE results for sample 2 is given in Figure 5.2.

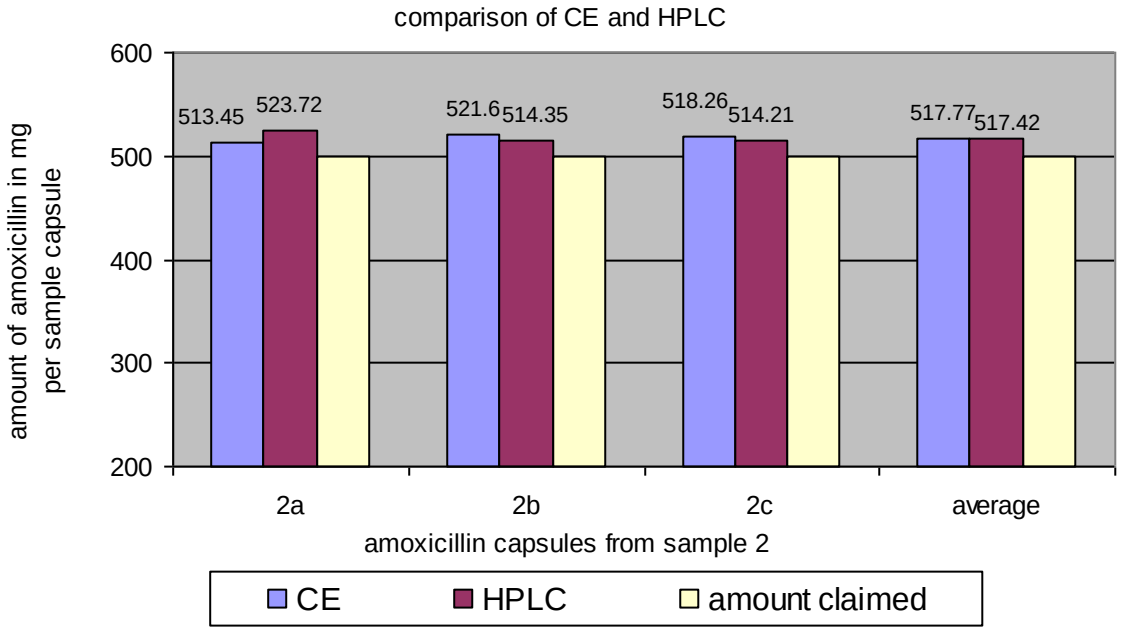


Figure.5.2: Comparison of CE and HPLC results for sample 2.

Figure 5.2 shows almost higher values of amoxicillin calculated per sample capsule by CE method compared to HPLC with exception of capsule 2a which shows higher value calculated by HPLC as compared to CE results. The reason for the difference between the HPLC and CE results has not been investigated at this level of study.

IR results:

For further conformation of contents, IR analysis of sample 2 was undertaken and the spectrum obtained was compared with the IR spectrum of pure amoxicillin. The various peaks observed are listed in Table 5.2b on the following page.

Table.5.2b: comparison between strong IR peaks of amoxicillin sample Werrimox and pure amoxicillin

S.No		λ Absorb.(max) $^{-1}$	λ Absorb.(max) $^{-2}$
1	Pure Amoxicillin	1686.32 cm^{-1}	1775.10 cm^{-1}
2	Sample 2a	1686.27 cm^{-1}	1775.13 cm^{-1}
3	Sample 2b	1686.63 cm^{-1}	1775.06 cm^{-1}
4	Sample 2c	1686.43 cm^{-1}	1774.84 cm^{-1}

The characteristic strong peaks observed at 1775.10 cm^{-1} and 1686.32 cm^{-1} were recorded in the IR spectrum of pure amoxicillin and amoxicillin sample Werrimox confirming the presence of –C=O in cyclic amide structure and –C=O in normal amine group respectively.

The rest of the medium and weak peaks observed in IR spectrum of sample 2 were also compared to those observed in IR spectrum of pure amoxicillin and were found the same.

Summary:

The retention and migration time recorded (from HPLC and CE respectively) for all the capsules from sample 2 agree the retention and migration time recorded for pure amoxicillin and also the IR spectra produced by capsules from sample 2 strongly correspond to the IR spectrum of pure amoxicillin. This confirms the presence of amoxicillin in sample 2.

The HPLC and CE analysis show that sample 2 contained relevant amount of amoxicillin more or less the exact amount stated by the manufacturer and meet the international standards of quality control thus did not show any counterfeiting.

Sample.3. Amoxicillin

CE results:

Three capsules from sample Amoxicillin were analysed and were designated as sample 3a, 3b and 3c.

Table.5.3: CE results produced by Amoxicillin sample capsules.

	Total weight of sample (mg)	Amoxicillin per capsule (as stated) (mg)	Migration Time (min)	amoxicillin per capsule (as sampled) (mg)	Number Of Repeats
3a	611	500	4.81	507.56	3
3b	600	500	4.71	531.72	3
3c	629	500	4.67	523	3
Average	**613.3**	**500.0**	**4.73**	**520.76**	**3.00**
St Dev	**11.95**	**0.0**	**0.07**	**9.98**	**0.00**

Each individual sample capsule was analysed three times and the standard deviation calculated for the migration time noted for each sample was almost equal to zero. This value of standard deviation suggests that repeatability exists within the sample capsules.

It was found that repeatability exists within the individual capsules but not between the capsules of the same brand because the amount of amoxicillin calculated per sample capsule is not the same throughout the samples and shows variation.

Table 5.3 shows that the amount (507.56 mg) calculated for amoxicillin in sample 3a, is slightly higher than the claimed quantity of 500 mg amoxicillin per sample capsule. However, sample capsule 3b and 3c show higher values as compared to the claimed amount of 500 mg amoxicillin per sample capsule.

The average amount of amoxicillin per sample capsule comes to be 520.76 mg

amoxicillin per sample capsule which is still a higher value as compared to the claimed amount but all these value are within the limits of international standards of quality control and therefore, the samples are of acceptable standard.

HPLC results:

HPLC results obtained from the analysis of capsules 3a, 3b and 3c from sample 3 (Amoxicillin) are listed in Table 4.3a.

Table.5.3a: HPLC results for sample Amoxicillin.

	Total weight of sample (mg)	Amoxicillin per capsule (as stated) (mg)	Retention Time (min)	Amoxicillin per capsule (as sampled) (mg)	Number Of Repeats
3a	611	500	4.69	509.43	3
3b	600	500	4.75	528.13	3
3c	629	500	4.77	519.74	3
Average	**613.33**	**500.0**	**4.74**	**519.1**	**3.00**
St Dev	**11.95**	**0.0**	**0.04**	**7.64**	**0.00**

All the three sample capsules were analysed three times repeatedly using HPLC under the same experimental conditions and the results recorded every time for each individual capsule were the same. This proved the individual capsules highly repeatable.

The results listed in Table 5.3a show that sample 3a contained a slightly higher amount of amoxicillin than the claimed quantity of 500 mg amoxicillin per sample capsule. Samples 3b and 3c are found to contain excess amount of amoxicillin compared to the claimed amount of 500 mg amoxicillin per sample capsule.

The average value of the individual calculated values of Amoxicillin per sample capsule comes to be 519.16 mg which is a higher value but all these value are

within the limits of international standards of quality control and therefore, the samples are of acceptable standard.

A chart is given in Figure 2.1.7 showing a comparison of CE and HPLC results for sample 3.

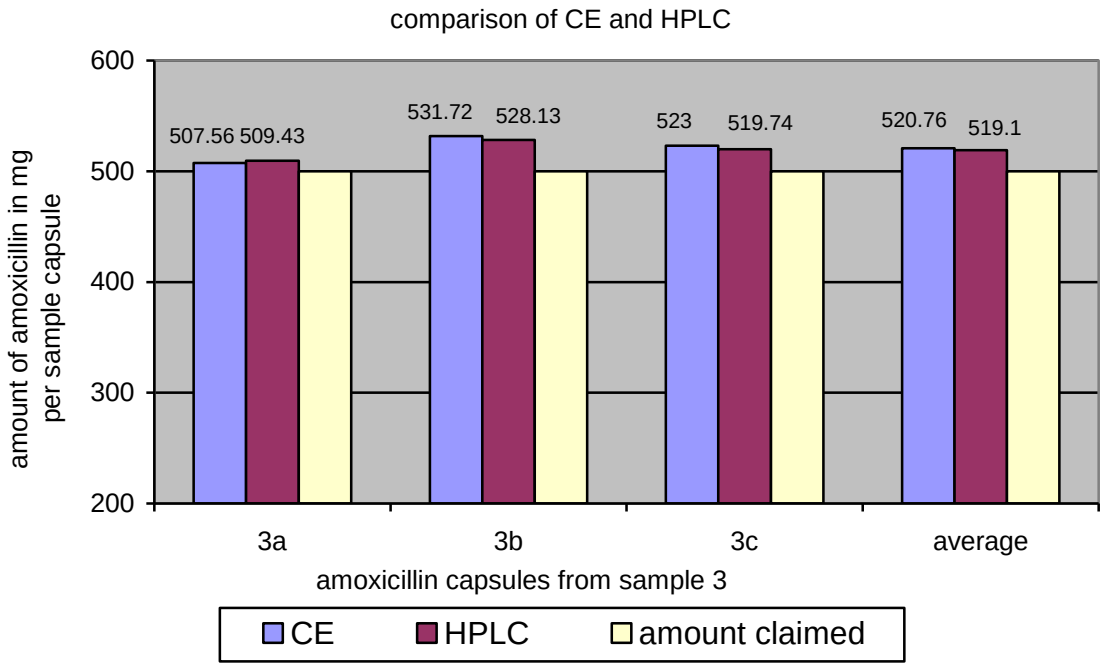


Figure.5.1.1: Bar chart representing comparison of HPLC and CE for sample 3.

Figure 5.1.1 shows almost higher values of amoxicillin calculated per sample capsule by CE method compared to HPLC with exception of capsule 3a which shows higher value calculated by HPLC as compared to CE results. The reason for any difference between the HPLC and CE results has not been investigated due to time limits for the project.

IR results:

For further conformation of contents, IR analysis of sample 3 was undertaken and the spectrum obtained was compared with the IR spectrum of pure amoxicillin. The various peaks observed are listed in Table 5.3b on the following page.

Table.5.3b: Comparison between strong IR peaks of amoxicillin sample amoxicillin and pure amoxicillin.

S.No		λ Absorb.(max) $^{-1}$	λ Absorb.(max) $^{-2}$
1	Pure Amoxicillin	1686.41 cm^{-1}	1775.03 cm^{-1}
2	Sample 3a	1686.43 cm^{-1}	1774.97 cm^{-1}
3	Sample 3b	1686.27 cm^{-1}	1775.23 cm^{-1}
4	Sample 3c	1686.26 cm^{-1}	1774.97 cm^{-1}

The characteristic strong peaks observed at 1775.03 cm^{-1} and 1686.41 cm^{-1} were recorded in the IR spectrum of pure amoxicillin and sample amoxicillin confirming the presence of ─C═O in cyclic amide structure and ─C═O in normal amine group respectively.

The rest of the medium and weak peaks observed in IR spectrum of sample 3 were also compared to those observed in IR spectrum of pure amoxicillin and were found the same.

Summary: The retention and migration time recorded (from HPLC and CE respectively) for all the capsules from sample 3 agree the retention and migration time recorded for pure amoxicillin and also the IR spectra produced by capsules from sample 3 strongly correspond to the IR spectrum of pure amoxicillin. This confirms the presence of amoxicillin in sample 3.

The HPLC and CE analysis show that sample 3 contained relevant amount of amoxicillin more or less the exact amount stated by the manufacturer and meet the international standard of quality control thus did not show any counterfeiting.

Sample.4. Effimox

CE results:

Three capsules from sample Effimox were analysed and were designated as sample 4a, 4b and 4c.

Table.5.4: CE results produced by Effimox sample capsules.

	Total weight of sample (mg)	Amoxicillin per capsule (as stated) (mg)	Migration Time (min)	Amoxicillin per capsule (as sampled) (mg)	Number Of Repeats
4a	593	500	4.69	509.64	3
4b	591	500	4.71	527	3
4c	618	500	4.74	523.76	3
Average	**600.7**	**500.0**	**4.71**	**520.1**	**3.00**
St Dev	**15.0**	**0.0**	**0.03**	**9.2**	**0.00**

Each individual sample capsule was analysed three times and the standard deviation calculated for migration time noted for each sample was almost equal to $\pm$ 0. This value of standard deviation suggests that repeatability exists within the sample capsules.

Furthermore, it is seen that these calculated amounts of amoxicillin in the sample capsules are not the same for all the capsules of the same brand. Therefore, it suggests that repeatability is found within the individual sample capsules but not between the capsules.

Table 5.4 shows that all the three Effimox sample capsule show higher values of amoxicillin as compared to claimed quantity of 500 mg amoxicillin per sample capsule.

The average calculated amount of amoxicillin per sample comes to be 520.12 mg

amoxicillin per sample capsule of Effimox which meets the international standards of quality control.

HPLC results:

HPLC results obtained from the analysis of capsules 4a, 4b and 4c from sample 4 (Effimox) are listed in Table 4.

Table.5.4a: HPLC results for sample Effimox.

	Total weight of sample (mg)	Amoxicillin per capsule (as stated) (mg)	Retention Time (min)	Amoxicillin per capsule (as sampled) (mg)	Number Of Repeats
4a	593	500	4.78	511.53	3
4b	591	500	4.81	519.67	3
4c	618	500	4.73	516.27	3
Average	**600.7**	**500.0**	**4.77**	**515.8**	**3.00**
St Dev	**15.0**	**0.0**	**0.04**	**3.33**	**0.00**

All the three sample capsules were analysed three times repeatedly using HPLC under the same experimental conditions and the results recorded every time for each individual capsule were the same. This proved the individual capsules highly repeatable.

Table 5.4a shows that all the three Effimox sample capsule show higher values of amoxicillin as compared to claimed quantity of 500 mg amoxicillin per sample capsule.

The average calculated amount of amoxicillin per sample comes to be 515.82 mg amoxicillin per sample capsule of Effimox which is a bit higher value of amoxicillin per sample capsule but is within the limits of international quality standards.

A comparison of CE and HPLC results is given in the Figure 2.1.8 on the following page.

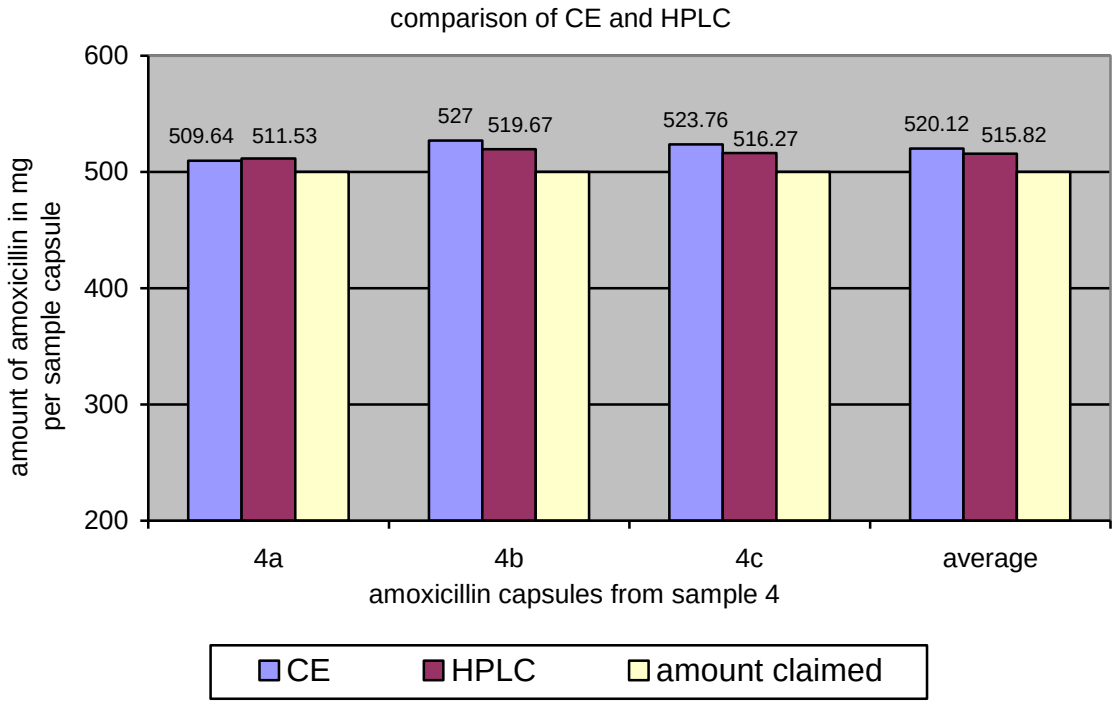


Figure.5.1.2: Bar chart representing comparison of CE and HPLC for sample 4.

Figure 5.1.2 shows almost higher values of amoxicillin calculated per sample capsule by CE method compared to HPLC with exception of capsule 4a which shows similar results produced by HPLC and CE analysis. The reason for the difference between the HPLC and CE results has not been investigated at this level of study.

IR results:

For further conformation of contents, IR analysis of sample 4 was undertaken and the spectrum obtained was compared with the IR spectrum of pure amoxicillin. The various peaks observed are listed in Table 5.4b on the following page.

Table.5.4b: Comparison between strong IR peaks of amoxicillin sample Effimox and pure amoxicillin

S.No		λ Absorb.(max) $^{-1}$	λ Absorb.(max) $^{-2}$
1	Pure Amoxicillin	1686.41 cm^{-1}	1775.10 cm^{-1}
2	Sample 4a	1686.43 cm^{-1}	1775.15 cm^{-1}
3	Sample 4b	1686.49 cm^{-1}	1774.96 cm^{-1}
4	Sample 4c	1686.26 cm^{-1}	1774.97 cm^{-1}

The characteristic strong peaks observed at 1775.10 cm^{-1} and 1686.41 cm^{-1} were recorded in the IR spectrum of pure amoxicillin and amoxicillin sample Effimox confirming the presence of –C=O in cyclic amide structure and –C=O in normal amine group respectively.

The rest of the medium and weak peaks observed in IR spectrum of sample 4 were also compared to those observed in IR spectrum of pure amoxicillin and were found the same.

Summary:

The retention and migration time recorded (from HPLC and CE respectively) for all the capsules from sample 4 agree the retention and migration time recorded for pure amoxicillin and also the IR spectra produced by capsules from sample 4 strongly correspond to the IR spectrum of pure amoxicillin. This confirms the presence of amoxicillin in sample 4.

The HPLC and CE analysis show that sample 4 contained relevant amount of amoxicillin more or less the exact amount stated by the manufacturer and meets the international standards of quality control thus did not show any counterfeiting.

Sample.5. Namoxil

CE results:

Three capsules from sample Namoxil were analysed and were designated as sample 5a, 5b and 5c.

Table.5.5: CE results produced by Namoxil sample capsules.

	Total weight of sample (mg)	Amoxicillin per capsule (as stated) (mg)	Migration Time (min)	Amoxicillin per capsule (as sampled) (mg)	Number Of Repeats
5a	572	500	4.95	511.78	3
5b	545	500	4.83	521.66	3
5c	655	500	4.96	528	3
Average	**590.7**	**500.0**	**4.91**	**520.5**	**3.00**
St Dev	**46.80**	**0.0**	**0.07**	**6.67**	**0.00**

Each individual sample capsule was analysed three times under the same set of experimental conditions and the amount calculated each time was the same. This repeated analysis of sample capsules proved that there is no variation of composition within the individual sample capsules and that they show a high degree of repeatability.

Table 5.5 shows that all the three Namoxil sample capsule show higher values of Amoxicillin as compared to claimed quantity of 500 mg Amoxicillin per sample capsule. The average calculated amount of Amoxicillin per sample comes to be 520.5 mg Amoxicillin per sample capsule of Namoxil which meets the international standards of quality control.

HPLC results:

HPLC results obtained from the analysis of capsules 5a, 5b and 5c from sample 5 (Namoxil) are listed in Table 4.5a given on the following page.

Table.5.5a: HPLC results for sample Namoxil.

	Total weight of sample (mg)	Amoxicillin per capsule (as stated) (mg)	Retention Time (min)	Amoxicillin per capsule (as sampled) (mg)	Number Of Repeats
5a	572	500	4.76	514.68	3
5b	545	500	4.75	521.97	3
5c	655	500	4.73	527.1	3
Average	**590.7**	**500.0**	**4.75**	**521.3**	**3.00**
St Dev	**46.80**	**0.0**	**0.02**	**5.09**	**0.00**

All the three sample capsules were analysed three times repeatedly using HPLC under the same experimental conditions and the results recorded every time for each individual capsule were the same. This proved the individual capsules highly repeatable.

Table 5.5a shows that all the three Namoxil sample capsules show higher values of Amoxicillin as compared to claimed quantity of 500 mg Amoxicillin per sample capsule. The average calculated amount of Amoxicillin per sample comes to be 521.3 mg Amoxicillin per sample capsule of Namoxil which meets the international standards of quality control.

A comparison of CE and HPLC is given in the Figure 5.1.3 on the following page.

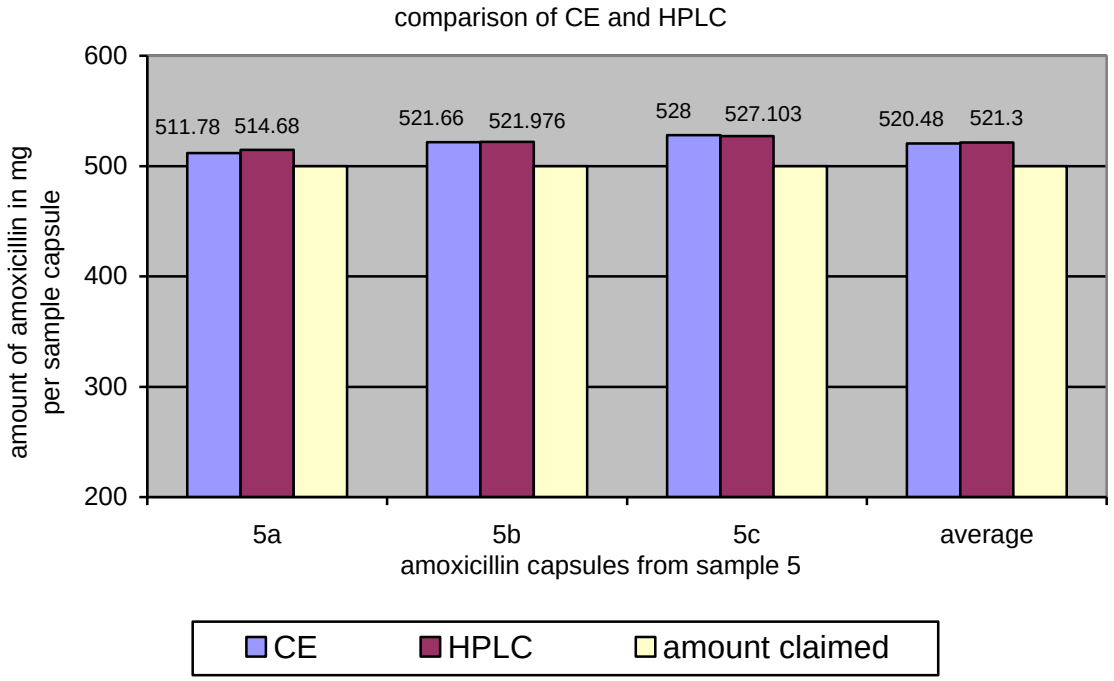


Figure.5.1.3: Bars showing comparison of CE and HPLC results for sample 5.

Figure 5.1.3 shows similar results for sample 5 as analysed by HPLC and CE techniques.

IR results:

For further conformation of contents, IR analysis of sample 11 was undertaken and the spectrum obtained was compared with the IR spectrum of pure amoxicillin. The various peaks observed are listed in Table 5.5b.

Table.5.5b: Comparison between strong IR peaks of amoxicillin sample Namoxil and pure amoxicillin.

S.No		λ Absorb.(max) $^{-1}$	λ Absorb.(max) $^{-2}$
1	Pure Amoxicillin	1686.32 cm^{-1}	1775.17 cm^{-1}
2	Sample 5a	1686.27 cm^{-1}	1775.13 cm^{-1}
3	Sample 5b	1686.63 cm^{-1}	1775.06 cm^{-1}
4	Sample 5c	1686.43 cm^{-1}	1774.84 cm^{-1}

The characteristic strong peaks observed at 1775.17 cm^{-1} and 1686.32 cm^{-1} were recorded in the IR spectrum of pure amoxicillin and amoxicillin sample Namoxil confirming the presence of ─C═O in cyclic amide structure and ─C═O in normal amine group respectively.

The rest of the medium and weak peaks observed in IR spectrum of sample 5 were also compared to those observed in IR spectrum of pure amoxicillin and were found the same.

Summary:

The retention and migration time recorded (from HPLC and CE respectively) for all the capsules from sample 5 agree the retention and migration time recorded for pure amoxicillin and also the IR spectra produced by capsules from sample 5 strongly correspond to the IR spectrum of pure amoxicillin. This confirms the presence of amoxicillin in sample 5.

The HPLC and CE analysis show that sample 5 contained relevant amount of amoxicillin more or less the exact amount stated by the manufacturer and meets the international standards of quality control thus did not show any counterfeiting.

Sample.6. Amoxascot

CE results:

Three capsules from sample Amoxascot were analysed and were designated as sample 6a, 6b and 6c.

Table.5.6: CE results produced by Amoxascot sample capsules.

	Total weight of sample (mg)	Amoxicillin per capsule (as stated) (mg)	Migration Time (min)	amoxicillin per capsule (as sampled) (mg)	number Of Repeats
6a	615	500	4.84	556.92	3
6b	611	500	4.79	558	3
6c	571	500	4.96	460	3
Average	**599.0**	**500.0**	**4.86**	**525.0**	**3.00**
St Dev	**19.86**	**0.0**	**0.09**	**45.94**	**0.00**

Each individual sample capsule was analysed three times under the same set of experimental conditions and the amount calculated each time was the same. This repeated analysis of sample capsules proved that there is no variation of composition within the individual sample capsules and that they show a high degree of repeatability.

The variation in content uniformity exists within the same brand and Table 5.6 shows that sample Amoxascot 6a and 6b contain more Amoxicillin than the claimed quantity of 500 mg Amoxicillin per sample capsule.

Sample capsule 6c contains less Amoxicillin as compared to the claimed amount of 500 mg of Amoxicillin per sample capsule. All the results are still within the limits of international standards of quality control.

HPLC results:

HPLC results obtained from the analysis of capsules 6a, 6b and 6c from sample

6 (Amoxascot) are listed in Table 4.6a.

Table.5.6a: HPLC results for sample Amoxascot.

	Total weight of sample (mg)	Amoxicillin per capsule (as stated) (mg)	Retention Time (min)	amoxicillin per capsule (as sampled) (mg)	Number Of Repeats
6a	615	500	4.95	558.92	3
6b	611	500	4.83	557.06	3
6c	571	500	4.96	458.94	3
Average	**599**	**500.0**	**4.91**	**525.0**	**3.00**
St Dev	**19.86**	**0.0**	**0.07**	**46.67**	**0.00**

All the three sample capsules were analysed three times repeatedly using HPLC under the same experimental conditions and the results recorded every time for each individual capsule were the same. This proved the individual capsules highly repeatable.

Table 5.6a shows that sample Amoxascot 6a and 6b contain more Amoxicillin than the claimed quantity of 500 mg Amoxicillin per sample capsule. Sample capsule 6c contains less amoxicillin as compared to the claimed amount of 500 mg of Amoxicillin per sample capsule.

The average calculated amount of Amoxicillin per sample capsule is 525 mg which is a higher value as compared to the claimed amount of 500 mg Amoxicillin per sample capsule. All these values meet the international standards of quality control.

A comparison of CE and HPLC results is given in the form of the Figure 5.1.4 on the following page.

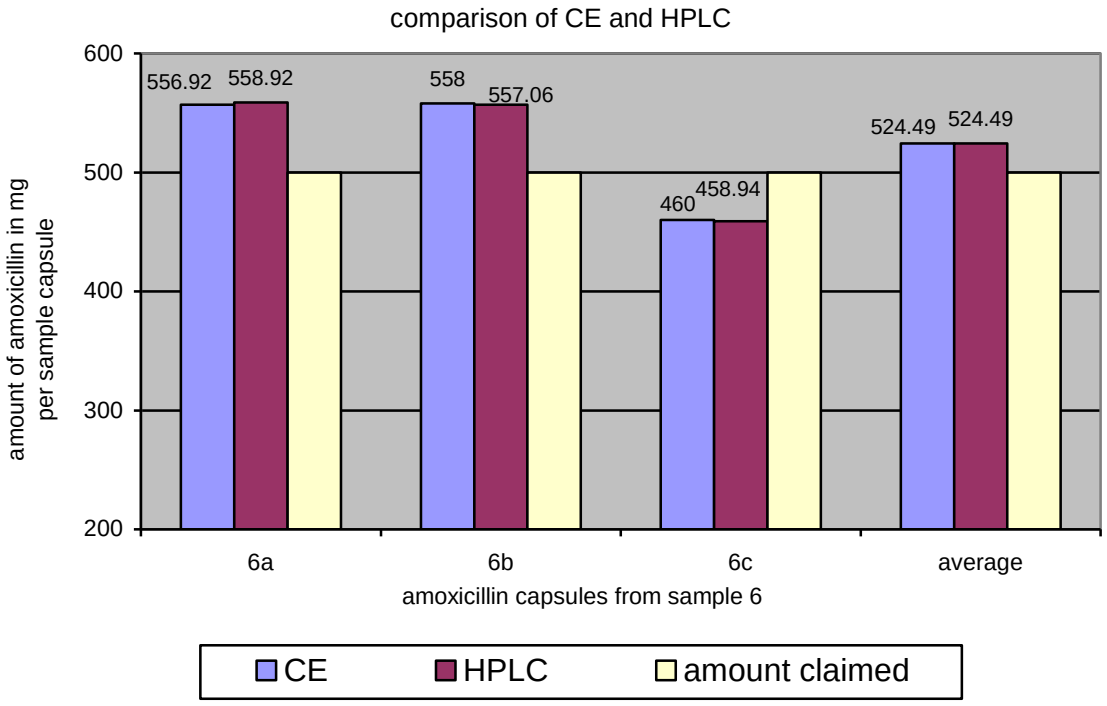


Figure.5.1.4: Bars showing comparison of CE and HPLC results for sample 6.

Figure 5.1.4 shows very similar results for sample 6 as produced from HPLC and CE analysis.

IR results:

For further conformation of contents, IR analysis of sample 6 was undertaken and the spectrum obtained was compared with the IR spectrum of pure amoxicillin. The various peaks observed are listed in Table 5.6b.

Table.5.6b: Comparison between strong IR peaks of amoxicillin sample Amoxascot and pure amoxicillin.

S.No		λ Absorb.(max) $^{-1}$	λ Absorb.(max) $^{-2}$
1	Pure Amoxicillin	1686.32 cm^{-1}	1775.10 cm^{-1}
2	Sample 6a	1686.29 cm^{-1}	1775.15 cm^{-1}
3	Sample 6b	1686.63 cm^{-1}	1775.03 cm^{-1}
4	Sample 6c	1686.47 cm^{-1}	1774.86 cm^{-1}

The characteristic strong peaks observed at 1775.10 cm^{-1} and 1686.32 cm^{-1} were recorded in the IR spectrum of pure amoxicillin and amoxicillin sample Amoxascot confirming the presence of ─C═O in cyclic amide structure and ─C═O in normal amine group respectively.

The rest of the medium and weak peaks observed in IR spectrum of sample 6 were also compared to those observed in IR spectrum of pure amoxicillin and were found the same.

Summary:

The retention and migration time recorded (from HPLC and CE respectively) for all the capsules from sample 6 agree the retention and migration time recorded for pure amoxicillin and also the IR spectra produced by capsules from sample 6 strongly correspond to the IR spectrum of pure amoxicillin. This confirms the presence of amoxicillin in sample 6.

The HPLC and CE analysis show that sample 6 contained relevant amount of amoxicillin more or less the exact amount stated by the manufacturer and meet the international standards of quality control thus did not show any counterfeiting.

Sample.7. Medimox

CE results:

Three capsules from sample Medimox were analysed and were designated as sample 7a, 7b and 7c.

Table.5.7: CE results produced by Medimox sample capsules.

	Total weight of sample (mg)	Amoxicillin per capsule (as stated) (mg)	migration Time (min)	amoxicillin per capsule (as sampled) (mg)	Number Of Repeats
7a	562	500	4.76	493.53	3
7b	602	500	4.75	517.38	3
7c	550	500	4.73	508.47	3
Average	**571.3**	**500.0**	**4.75**	**506.5**	**3.00**
St Dev	**22.23**	**0.0**	**0.02**	**9.83**	**0.00**

Each individual sample capsule was analysed three times under the same set of experimental conditions and the amount calculated each time was the same. This repeated analysis of sample capsules proved that there is no variation of composition within the individual sample capsules and that they show a high degree of repeatability.

However, the amounts of active ingredients found in all the samples are not the same and a variation in content uniformity exists within the same brand.

Capsule 7b and 7c show are found to contain higher values of amoxicillin per capsule as compared to the claimed amount of 500 mg Amoxicillin per sample capsule. The average amount of amoxicillin per sample capsule comes to be 506.4 mg which is slightly higher than the mentioned or claimed quantity and meets the international standards of quality control.

HPLC results:

HPLC results obtained from the analysis of capsules 7a, 7b and 7c from sample 7 (Medimox) are listed in Table 4.7a on the next page.

Table.5.7a: HPLC results for sample Medimox.

	Total weight of sample (mg)	Amoxicillin per capsule (as stated) (mg)	Retention Time (min)	amoxicillin per capsule (as sampled) (mg)	Number Of Repeats
7a	562	500	4.84	496.47	3
7b	602	500	4.76	519.44	3
7c	550	500	4.79	503.57	3
Average	**571.3**	**500.0**	**4.80**	**506.5**	**3.00**
St Dev	**22.23**	**0.0**	**0.04**	**9.60**	**0.00**

All the three sample capsules were analysed three times repeatedly using HPLC under the same experimental conditions and the results recorded every time for each individual capsule were the same. This proved the individual capsules highly repeatable.

Table 5.7a shows that the sample capsule Medimox 7a contains less amoxicillin as compared to the claimed quantity of 500 mg Amoxicillin per sample capsule. Capsule 7b and 7c show are found to contain higher values of amoxicillin per capsule as compared to the claimed amount of 500 mg Amoxicillin per sample capsule.

The average amount of amoxicillin per sample capsule comes to be 506.4 mg which is slightly higher than the mentioned or claimed quantity but is in the limit of international standards of quality control.

A comparison of CE and HPLC results for sample 7 is given in the Figure 5.1.5. on the following page.

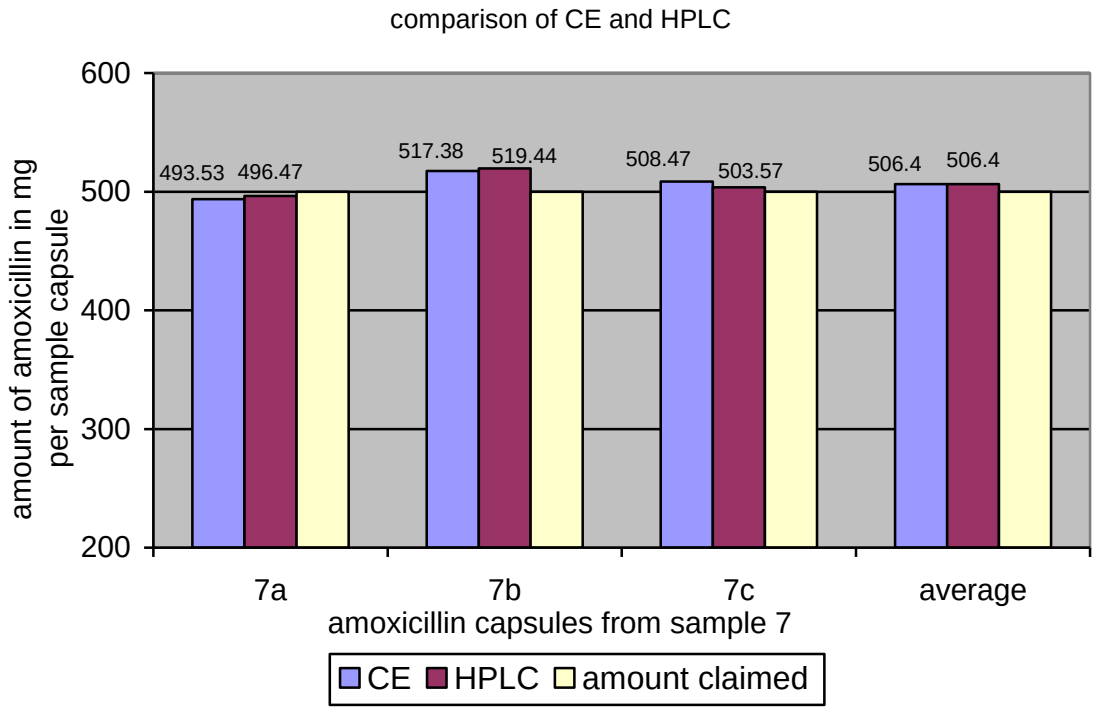


Figure.5.1.5: Bars showing comparison of CE and HPLC results for sample 7.

Figure 5.1.5 shows that the HPLC and CE results obtained from the analysis of sample 7 are nearly the same with small differences for capsules 7a (CE value slightly lower than HPLC) and capsule 7c (CE value slightly higher than HPLC). The reason for the difference between the HPLC and CE results has not been investigated at this level of study.

IR results: For further conformation of contents, IR analysis of sample 7 was undertaken and the spectrum obtained was compared with the IR spectrum of pure amoxicillin. The various peaks observed are listed in Table 5.7b on the following page.

Table.5.7b: Comparison between strong IR peaks of amoxicillin sample Medimox and pure amoxicillin.

S.No		λ Absorb.(max) $^{-1}$	λ Absorb.(max) $^{-2}$
1	Pure Amoxicillin	1686.32 cm^{-1}	1775.10 cm^{-1}
2	Sample 7a	1686.27 cm^{-1}	1775.13 cm^{-1}
3	Sample 7b	1686.63 cm^{-1}	1775.06 cm^{-1}
4	Sample 7c	1686.43 cm^{-1}	1774.84 cm^{-1}

The characteristic strong peaks observed at 1775.10 cm^{-1} and 1686.32 cm^{-1} were recorded in the IR spectrum of pure amoxicillin and amoxicillin sample Medimox confirming the presence of –C=O in cyclic amide structure and –C=O in normal amine group respectively.

The rest of the medium and weak peaks observed in IR spectrum of sample 7 were also compared to those observed in IR spectrum of pure amoxicillin and were found the same.

Summary: The retention and migration time recorded (from HPLC and CE respectively) for all the capsules from sample 7 agree the retention and migration time recorded for pure amoxicillin and also the IR spectra produced by capsules from sample 7 strongly correspond to the IR spectrum of pure amoxicillin. This confirms the presence of amoxicillin in sample 7.

The HPLC and CE analysis show that sample 7 contained relevant amount of amoxicillin more or less the exact amount stated by the manufacturer and meets the international standards of quality control thus did not show any counterfeiting.

Sample.8. Labmox

CE results:

Three capsules from sample Labmox were analysed and were designated as sample 8a, 8b and 8c.

Table.5.8: CE results produced by Labmox sample capsules.

	Total weight of sample (mg)	Amoxicillin per capsule (as stated) (mg)	migration Time (min)	Amoxicillin per capsule (as sampled) (mg)	Number Of Repeats
8a	565	500	4.7	509.58	3
8b	580	500	4.7	496.43	3
8c	551	500	4.69	512.83	3
Average	**565.3**	**500.0**	**4.70**	**506.28**	**3.00**
St Dev	**11.84**	**0.0**	**0.01**	**7.09**	**0.00**

Each individual sample capsule was analysed three times under the same set of experimental conditions and the amount calculated each time was the same. This repeated analysis of sample capsules proved that there is no variation of composition within the individual sample capsules and that they show a high degree of repeatability.

Table 5.8 shows that the sample capsule 8b is found to have slightly lower value of amoxicillin as the claimed amount of 500 mg amoxicillin per sample capsule, while sample capsules 8a and 8c show higher amounts of amoxicillin above the claimed amount of 500 mg amoxicillin per sample capsule.

The average calculated value of amoxicillin per capsule is found to be 506.28 mg which is lower than the claimed amount of 500 mg amoxicillin / sample capsule. The results produced by sample 8 meet the international standards of quality control.

HPLC results:

HPLC results obtained from the analysis of capsules 8a, 8b and 8c from sample 8 (Labmox) are listed in Table 4.8a.

Table.5.8a: HPLC results for sample Labmox.

	Total weight of sample (mg)	Amoxicillin per capsule (as stated) (mg)	Retention Time (min)	amoxicillin per capsule (as sampled) (mg)	number Of repeats
8a	565	500	4.77	516.83	3
8b	580	500	4.73	498.73	3
8c	551	500	4.76	507.64	3
Average	**565.3**	**500.0**	**4.75**	**507.7**	**3.00**
St Dev	**11.84**	**0.0**	**0.02**	**7.38**	**0.00**

All the three sample capsules were analysed three times repeatedly using HPLC under the same experimental conditions and the results recorded every time for each individual capsule were the same. This proved the individual capsules highly repeatable.

Table 5.8a shows that the sample Labmox capsule 8b is found to have very slightly lower value of Amoxicillin than the claimed amount of 500 mg Amoxicillin per sample capsule, while sample capsules 8a and 8c show higher amount of Amoxicillin above the claimed amount of 500 mg Amoxicillin per sample capsule.

The average calculated value of Amoxicillin per capsule is found to be 507.73 mg which is higher than the claimed amount of 500 mg Amoxicillin / sample capsule. All the results produced by sample 8 are according to international standards of quality control.

A comparison of CE and HPLC results is given in the Figure 5.1.6 on the following page.

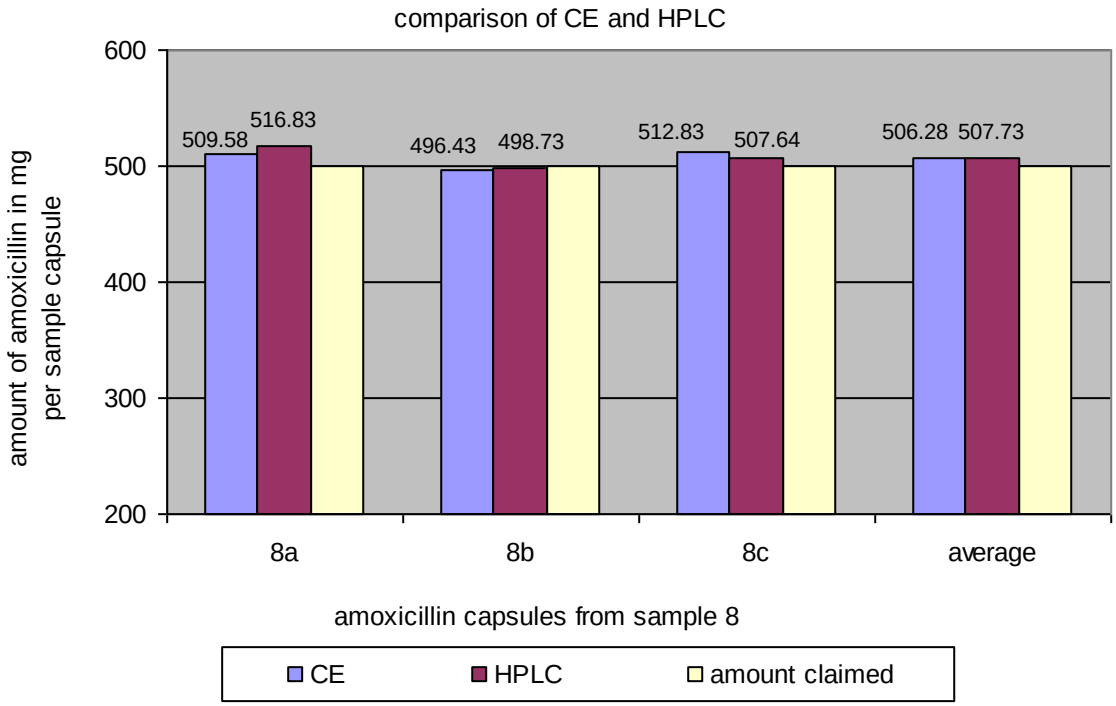


Figure.5.1.6: Bar chart showing comparison of CE and HPLC results for sample 8.

Figure 5.1.6 indicates higher values of amoxicillin calculated HPLC compared to CE for capsules 8b and 8c, while CE results for capsule 8a are lower as compared to HPLC results and the average value calculated for CE and HPLC is found to be the same. The reason for any difference between the HPLC and CE results has not been investigated due to time limits for the project.

IR results:

For further conformation of contents, IR analysis of sample 8 was undertaken and the spectrum obtained was compared with the IR spectrum of pure amoxicillin. The various peaks observed are listed in Table 5.8b on the following page.

Table.5.8b: Comparison between strong IR peaks of amoxicillin sample Labmox and pure amoxicillin.

S.No		λ Absorb.(max) $^{-1}$	λ Absorb.(max) $^{-2}$
1	Pure Amoxicillin	1686.32 cm^{-1}	1775.11 cm^{-1}
2	Sample 8a	1686.24 cm^{-1}	1775.17 cm^{-1}
3	Sample 8b	1686.61 cm^{-1}	1775.09 cm^{-1}
4	Sample 8c	1686.45 cm^{-1}	1774.81 cm^{-1}

The characteristic strong peaks observed at 1775.11 cm^{-1} and 1686.32 cm^{-1} were recorded in the IR spectrum of pure amoxicillin and amoxicillin sample Labmox confirming the presence of ─C═O in cyclic amide structure and ─C═O in normal amine group respectively.

The rest of the medium and weak peaks observed in IR spectrum of sample 8 were also compared to those observed in IR spectrum of pure amoxicillin and were found the same.

Summary:

The retention and migration time recorded (from HPLC and CE respectively) for all the capsules from sample 8 agree the retention and migration time recorded for pure amoxicillin and also the IR spectra produced by capsules from sample 8 strongly correspond to the IR spectrum of pure amoxicillin. This confirms the presence of amoxicillin in sample 8.

The HPLC and CE analysis show that sample 8 contained relevant amount of amoxicillin more or less the exact amount stated by the manufacturer and meets the international standards of quality control thus did not show any counterfeiting.

Sample .9. HMC

CE results:

Three capsules from sample HMC were analysed and were designated as sample 9a, 9b and 9c.

Table.5.9: CE results produced by HMC sample capsules.

	Total weight of sample (mg)	Amoxicillin per capsule (as stated) (mg)	migration Time (min)	Amoxicillin per capsule (as sampled) (mg)	Number Of Repeats
9a	296	250	4.65	254.63	3
9b	307	250	4.64	256.86	3
9c	293	250	4.63	248.65	3
Average	**298.7**	**250.0**	**4.64**	**253.38**	**3.00**
St Dev	**6.01**	**0.0**	**0.01**	**3.46**	**0.00**

Each individual sample capsule was analysed three times under the same set of experimental conditions and the amount calculated each time was the same. This repeated analysis of sample capsules proved that there is no variation of composition within the individual sample capsules and that they show a high degree of repeatability.

Table 5.9 shows that capsule 9a from sample HMC is found to contain a slightly higher value of amoxicillin than the claimed amount of 250 mg amoxicillin per capsule.

Sample capsule 9c is found to contain slightly less Amoxicillin than the claimed quantity while sample capsule 9b is found to contain 256.86 mg amoxicillin which is slightly higher than the claimed amount of 250 mg amoxicillin per sample capsule. The average calculated amount of amoxicillin is 253.38 mg per sample capsule.

Each individual capsule show a good standard of repeatability but it is found that the amount of amoxicillin is not the same in all the sample capsules analysed. A variation in content uniformity is found within the same brand of amoxicillin.

HPLC results:

HPLC results obtained from the analysis of capsules 9a, 9b and 9c from sample 9 (HMC) are listed in Table 4.9a.

Table.5.9a: HPLC results for sample HMC.

	Total weight of sample (mg)	Amoxicillin per capsule (as stated) (mg)	Retention Time (min)	Amoxicillin per capsule (as sampled) (mg)	Number Of Repeats
9a	296	250	4.78	257.84	3
9b	307	250	4.78	261.73	3
9c	293	250	4.76	253.63	3
Average	**298.7**	**250**	**4.77**	**257.7**	**3.00**
St Dev	**6.01**	**0.0**	**0.01**	**3.3**	**0.00**

All the three sample capsules were analysed three times repeatedly using HPLC under the same experimental conditions and the results recorded every time for each individual capsule were the same. This proved the individual capsules highly repeatable.

Table 5.9a shows that all the three HMC sample capsules show higher values of amoxicillin as compared to claimed quantity of 500 mg amoxicillin per sample capsule.

The average calculated amount of Amoxicillin per sample comes to be 257.73 mg Amoxicillin per sample capsule of HMC which meets the international standards of quality control.

The comparison of CE and HPLC results is given in the Figure 5.1.7.

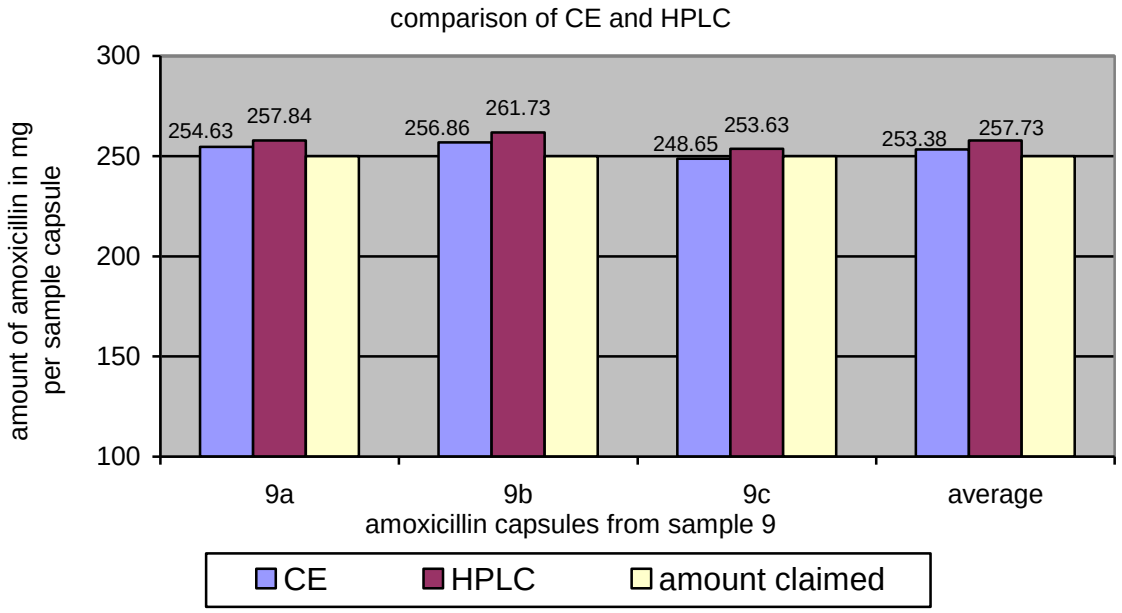


Figure.5.1.7: Bars showing comparison of CE and HPLC results for sample 9.

Figure 5.1.7 shows that, for the HPLC analysis of sample 9, the calculated values of amoxicillin per sample capsule are higher for all the sample capsules as compared to the values calculated by using CE technique. The reason for the difference between the HPLC and CE results has not been fully investigated due to time limits for the project.

IR results:

For further conformation of contents, IR analysis of sample 9 was undertaken and the spectrum obtained was compared with the IR spectrum of pure amoxicillin. The various peaks observed are listed in Table 5.9b on the following page.

Table.5.9b: Comparison between strong IR peaks of amoxicillin sample HMC and pure amoxicillin.

S.No		λ Absorb.(max) $^{-1}$	λ Absorb.(max) $^{-2}$
1	Pure Amoxicillin	1686.32 cm^{-1}	1775.10 cm^{-1}
2	Sample 9a	1686.27 cm^{-1}	1775.13 cm^{-1}
3	Sample 9b	1686.63 cm^{-1}	1775.06 cm^{-1}
4	Sample 9c	1686.43 cm^{-1}	1774.84 cm^{-1}

The characteristic strong peaks observed at 1775.10 cm^{-1} and 1686.32 cm^{-1} were recorded in the IR spectrum of pure amoxicillin and amoxicillin sample HMC confirming the presence of ─C═O in cyclic amide structure and ─C═O in normal amine group respectively.

The rest of the medium and weak peaks observed in IR spectrum of sample 9 were also compared to those observed in IR spectrum of pure amoxicillin and were found the same.

Summary:

The retention and migration time recorded (from HPLC and CE respectively) for all the capsules from sample 9 agree the retention and migration time recorded for pure amoxicillin and also the IR spectra produced by capsules from sample 9 strongly correspond to the IR spectrum of pure amoxicillin. This confirms the presence of amoxicillin in sample 9.

The HPLC and CE analysis show that sample 9 contained relevant amount of amoxicillin more or less the exact amount stated by the manufacturer and meets the international standards of quality control thus did not show any counterfeiting.

Sample .10. Acamoxil

CE results:

Three capsules from sample Acamoxil were analysed and were designated as sample 10a, 10b, and 10c.

Table.5.1.1: CE results produced by Acamoxil sample capsules.

	Total weight of sample (mg)	Amoxicillin per capsule (as stated) (mg)	migration time (min)	Amoxicillin per capsule (as sampled) (mg)	Number Of Repeats
10a	278	250	No peak	0	3
10b	284	250	4.67	235.57	3
10c	282	250	4.65	257.63	3
10d	289	250	4.65	247.98	3
Average	**283.3**	**250.0**	**4.66**	**247.06**	**3.00**
St Dev	**3.96**	**0.0**	**0.01**	**9.02**	**0.00**

Each individual sample capsule was analysed three times under the same set of experimental conditions and the amount calculated each time was the same. This repeated analysis of sample capsules proved that there is no variation of composition within the individual sample capsules and that they show a high degree of repeatability.

Table 5.1.1 shows that Acamoxil sample capsule 10b is found to contain less amoxicillin than the claimed amount of 250 mg amoxicillin per sample capsule but still meets the international standards of quality control.

The sample capsule 10a is found to contain no amoxicillin at all. These results were confirmed by analysing the contents of sample capsule 10a repeatedly for five times under the same experimental conditions. The results produced were

the same having no Amoxicillin peaks. The electropherogram obtained for sample capsule 10a was similar to the electropherogram produced by the analysis of pure water (HPLC grade water) only. This confirmed that sample capsule 10a did not contain any active ingredients and was a substandard or counterfeit sample.

Further investigations were made by analysing another capsule from the same sample, designated as 10d. This sample capsule produced the same results as sample 10b and 10c confirming that sample capsule 10a did not contain any amoxicillin. The CE electropherograms produced by HPLC grade water and sample capsule
10a are similar and are given in the Figures 5.2.7 and 5.2.8.

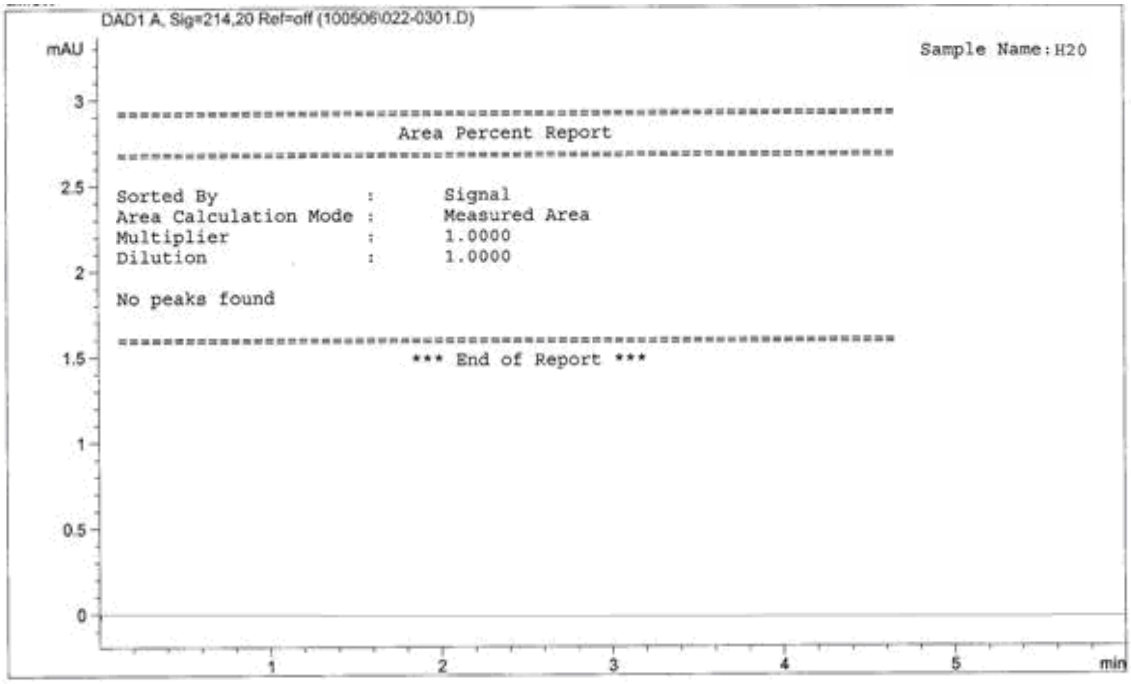


Figure.5.2.7: CE electropherogram for HPLC H_2O. NO peak was observed for pure HPLC grade water.

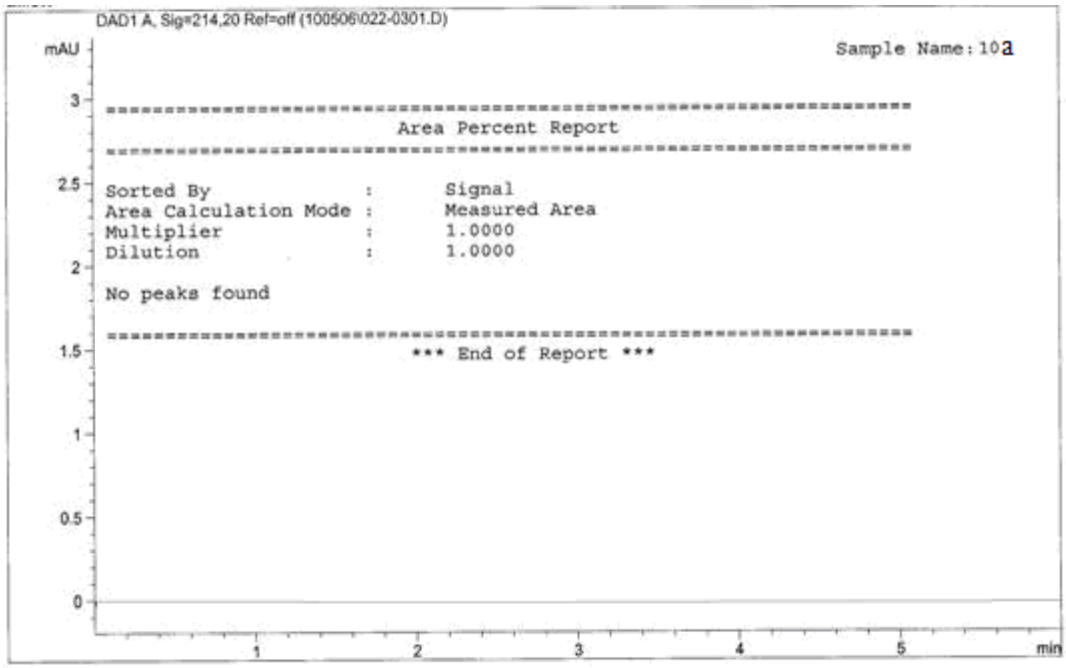


Figure.5.2.8: CE electropherogram for sample capsule 10a. No peak was observed for sample 10 a.

HPLC results:

HPLC results obtained from the analysis of capsules 10a, 10b and10c from sample10 (Acamoxil) are listed in Table 4.1.1a.

Table.5.1.1a: HPLC results.

	Total weight of sample (mg)	Amoxicillin per capsule (as stated) (mg)	Retention time (min)	Amoxicillin per capsule (as sampled) (mg)	Number Of Repeats
10a	278	250	No peak	0	3
10b	284	250	4.77	237.87	3
10c	282	250	4.73	258.21	3
10d	289	250	4.76	246.76	3
Average	**283.3**	**250.0**	**4.75**	**247.61**	**3.00**
St Dev	**3.96**	**0.0**	**0.03**	**8.32**	**0.00**

All the four sample capsules were analysed three times repeatedly using HPLC

under the same experimental conditions and the results recorded every time for each individual capsule were the same. This proved the individual capsules highly repeatable.

By studying Table 5.1.1a it is clear that no peak was recorded for sample 10a and suggests containing no amoxicillin. Sample10b is found to contain less amount of amoxicillin per sample capsule as compared to the claimed amount of 250 mg per sample capsule. Sample 10c shows a slightly higher value of amoxicillin (but meets the international standards of quality control), while sample 10d produces the results that are very close to the claimed quantity of 250 mg amoxicillin per sample capsule.

The average value of the calculated amounts is 247.61 mg amoxicillin per sample capsule. This value is a very low calculated amount as compared to the amount of 250 mg amoxicillin per sample capsule as mentioned or claimed by the manufacturer.

A comparison of CE and HPLC results for sample 10 is given in the Figure 5.1.8 on the following page.

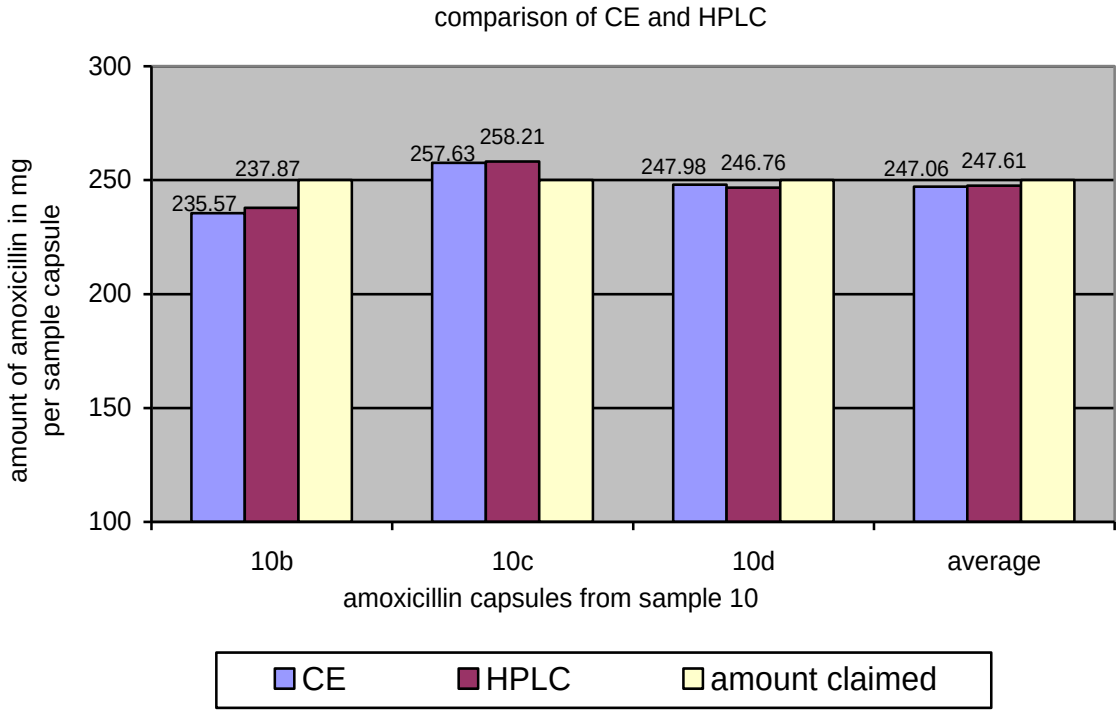


Figure.5.1.8: Bars showing comparison of CE and HPLC results for sample 10.

Figure 5.1.8 shows that, the results produced by sample 10 using HPLC and CE are almost the same for all the capsules with only minor differences. The reason for the difference between the HPLC and CE results has not been investigated due to time limits for the project.

IR results:

For further conformation of contents, IR analysis of sample 11 was undertaken and the spectrum obtained was compared with the IR spectrum of pure amoxicillin. The various peaks observed are listed in Table 5.1.2b on the following page.

Table.5.1.1b: Comparison between strong IR peaks of amoxicillin sample Acamoxil and pure amoxicillin.

S.No		λ Absorb.(max) $^{-1}$	λ Absorb.(max) $^{-2}$
1	Pure amoxicillin	1686.32 cm^{-1}	1775.10 cm^{-1}
2	Sample 10b	1686.27 cm^{-1}	1775.13 cm^{-1}
3	Sample 10c	1686.63 cm^{-1}	1775.06 cm^{-1}
4	Sample 10d	1686.43 cm^{-1}	1774.84 cm^{-1}

The characteristic strong peaks observed at 1775.10 cm^{-1} and 1686.32 cm^{-1} were recorded in the IR spectrum of pure amoxicillin and amoxicillin sample Acamoxil confirming the presence of –C=O in cyclic amide structure and –C=O in normal amine group respectively. The rest of the medium and weak peaks observed in IR spectrum of sample 10 were also compared to those observed in IR spectrum of pure amoxicillin and were found the same.

Summary:

The retention and migration time recorded (from HPLC and CE respectively) for all the capsules from sample 10 agree the retention and migration time recorded for pure amoxicillin and also the IR spectra produced by capsules from sample 10 strongly correspond to the IR spectrum of pure amoxicillin. This confirms the presence of amoxicillin in sample 10.

However, the HPLC and CE analysis show that one capsule from sample 10 did not contained any amoxicillin and was found blank. The average amount of amoxicillin per sample capsule was very less as compared to the stated amount of 250 mg amoxicillin per capsule. These results suggest sample 10 as counterfeit and substandard. As all other sample capsules from sample 10 contained the relative exact amount of amoxicillin stated by the manufacturer, therefore, it follows the possibility for sample 10 to be counterfeited along the distribution chain (may have been repacked or one of the original capsules may have been replaced with a blank capsule without any active ingredients).

Sample .11. Glomox

CE results:

Three capsules from sample Glomox were analysed and were designated as sample 11a, 11b and 11c.

Table.5.1.2: CE results produced by Glomox sample capsules.

	Total weight of sample (mg)	Amoxicillin per capsule (as stated) (mg)	migration time (min)	Amoxicillin per capsule (as sampled) (mg)	Number Of Repeats
11a	549	500	4.62	523.64	3
11b	598	500	4.65	518.45	3
11c	566	500	4.55	522.82	3
Average	**571.0**	**500.0**	**4.61**	**521.6**	**3.00**
St Dev	**20.31**	**0.0**	**0.05**	**2.27**	**0.00**

Each individual sample capsule was analysed three times under the same set of experimental conditions and the amount calculated each time was the same. This repeated analysis of sample capsules proved that there is no variation of composition within the individual sample capsules and that they show a high degree of repeatability.

Table 5.1.2 shows that all the three Glomox sample capsule show slightly higher values of amoxicillin as compared to claimed quantity of 500 mg amoxicillin per sample capsule. The average calculated amount of amoxicillin per sample comes to be 521.64 mg amoxicillin per sample capsule of Glomox which meets the international standards of quality control.

HPLC results:

HPLC results obtained from the analysis of capsules 11a, 11b and 11c from sample11 (Glomox) are listed in Table 4.1.2a on the following page.

Table.5.1.2a: HPLC results for sample Glomox.

	Total weight of sample (mg)	Amoxicillin per capsule (as stated) (mg)	Retention time (min)	amoxicillin per capsule (as sampled) (mg)	Number Of Repeats
11a	549	500	4.81	515.32	3
11b	598	500	4.78	517.73	3
11c	566	500	4.74	517.43	3
Average	**571.0**	**500.0**	**4.78**	**516.8**	**3.00**
St Dev	**20.31**	**0.0**	**0.04**	**1.07**	**0.00**

All the three sample capsules were analysed three times repeatedly using HPLC under the same experimental conditions and the results recorded every time for each individual capsule were the same. This proved the individual capsules highly repeatable.

Table 5.1.2a shows that all the three Glomox sample capsule show slightly higher values of amoxicillin as compared to claimed quantity of 500 mg amoxicillin per sample capsule. The average calculated amount of amoxicillin per sample comes to be 516.82 mg amoxicillin per sample capsule of Glomox which meets the international standards of quality control and hence the sample is of good quality and is not found to be counterfeited.

A comparison of CE and HPLC results is given in the Figure 5.1.9 on the following page.

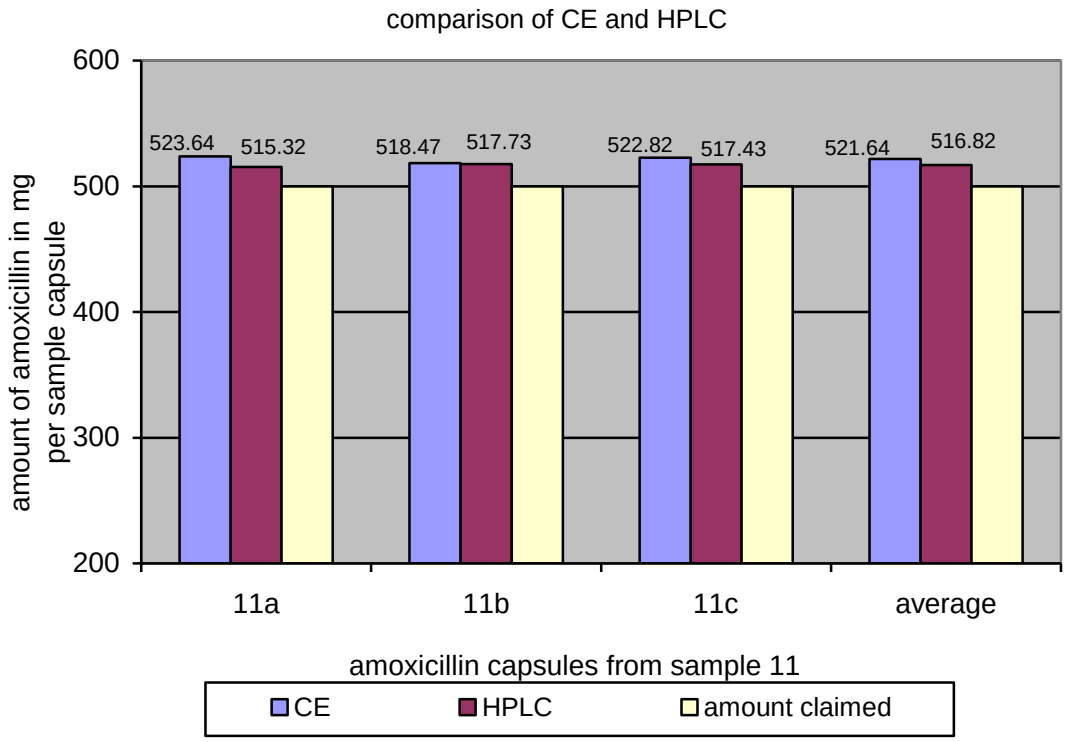


Figure.5.1.9: Bars showing comparison of CE and HPLC results for sample 11.

Figure 5.1.9 shows higher calculated values of amoxicillin per sample capsule using CE as compared to the HPLC analysis of the same sample (sample 11) with the exception of sample capsule 11b showing the same CE and HPLC results. The reason for the difference between the HPLC and CE results has not been fully investigated due to the time limits for the project.

IR results:

For further conformation of contents, IR analysis of sample 11 was undertaken and the spectrum obtained was compared with the IR spectrum of pure amoxicillin. The various peaks observed are listed in Table 5.1.2b on the following page.

Table.5.1.2b: Comparison between strong IR peaks of amoxicillin sample Glomox and pure amoxicillin.

S.No		λ Absorb.(max)$^{-1}$	λ Absorb.(max) $^{-2}$
1	Pure Amoxicillin	1686.32 cm^{-1}	1775.10 cm^{-1}
2	Sample 11a	1686.27 cm^{-1}	1775.13 cm^{-1}
3	Sample 11b	1686.63 cm^{-1}	1775.06 cm^{-1}
4	Sample 11c	1686.43 cm^{-1}	1774.84 cm^{-1}

The characteristic strong peaks observed at 1775.10 cm^{-1} and 1686.32 cm^{-1} were recorded in the IR spectrum of pure amoxicillin and compared with IR spectrum of amoxicillin sample Glomox confirming the presence of ─C═O in cyclic amide structure and ─C═O in normal amine group respectively.

The rest of the medium and weak peaks observed in IR spectrum of sample 11 were also compared to those observed in IR spectrum of pure amoxicillin and were found the same.

Summary:

The retention and migration time recorded (from HPLC and CE respectively) for all the capsules from sample11 agree the retention and migration time recorded for pure amoxicillin and also the IR spectra produced by capsules from sample 11 strongly correspond to the IR spectrum of pure amoxicillin. This confirms the presence of amoxicillin in sample 11.

The HPLC and CE analysis show that sample 11 contained relevant amount of amoxicillin more or less the exact amount stated by the manufacturer and meets the international standards of quality control thus did not show any counterfeiting.

Comparison of results from HPLC and CE analysis of all the amoxicillin samples.

The average or mean values of the individual calculated amounts for all Amoxicillin samples are compared for HPLC and CE analysis and are listed below.

Table.5.1.3: Comparison of average amounts of all the samples as calculated from HPLC and CE analysis.

Sample	HPLC results (mg)	CE results (mg)	Amount stated (mg)	Difference ±	average (mg)	STDEV ±
Maxil	530.57	529.69	500	0.88	530.13	0.11
Werrimox	517.42	518.57	500	1.15	517.99	0.57
Amoxicillin	519.16	520.76	500	1.6	519.96	0.8
Effimox	515.82	520.12	500	4.3	517.97	2.15
Namoxil	521.3	520.48	500	0.82	520.89	0.41
Amoxascot	524.49	524.49	500	0	524.49	0
Medimox	506.4	506.4	500	0	506.4	0
Labmox	507.73	506.28	500	1.45	507.0	0.72
Glomox	516.82	521.64	500	4.82	519.23	2.41
HMC	257.73	253.38	250	4.35	255.56	2.17
Acamoxil	185.71	185.29	250	0.42	185.5	0.21

All the average calculated amounts listed in the above Table are the amounts of amoxicillin per sample capsule for each brand of amoxicillin analysed by HPLC and capillary electrophoresis. Observation of Table 5.1.3 shows that the average calculated amounts of amoxicillin for most of the samples analysed by HPLC are slightly different than average calculated amounts as a result of their CE analysis.

Some of the samples have almost or exactly the same average values of calculated amounts of amoxicillin per sample, by both the HPLC and CE

analysis, such as sample Namoxil, Amoxascot and Medimox show the same values of average calculated amounts of Amoxicillin per sample capsule when analysed by both HPLC and CE techniques. Similarly sample Werrimox produces almost the same results by both HPLC and CE analysis with a small difference of $\pm$ 1.15 mg.

The reason for the differences found between the HPLC and CE results has not been investigated due to limited time frame for the project.

5.1: Quantitative comparison of different brands of amoxicillin samples:

The CE and HPLC results for all the different brands were compared separately such that the CE results are compared for all the samples and the HPLC results are compared for all the samples. All the CE and HPLC results are listed in form of comparison charts.

A comparison of results for all the amoxicillin samples analysed is given in the Figures 5.3.5, 5.3.7 (for samples containing 500 mg amoxicillin per capsule) and 5.3.6 and 5.3.8 (for samples containing 250 mg amoxicillin per capsule) on the following pages.

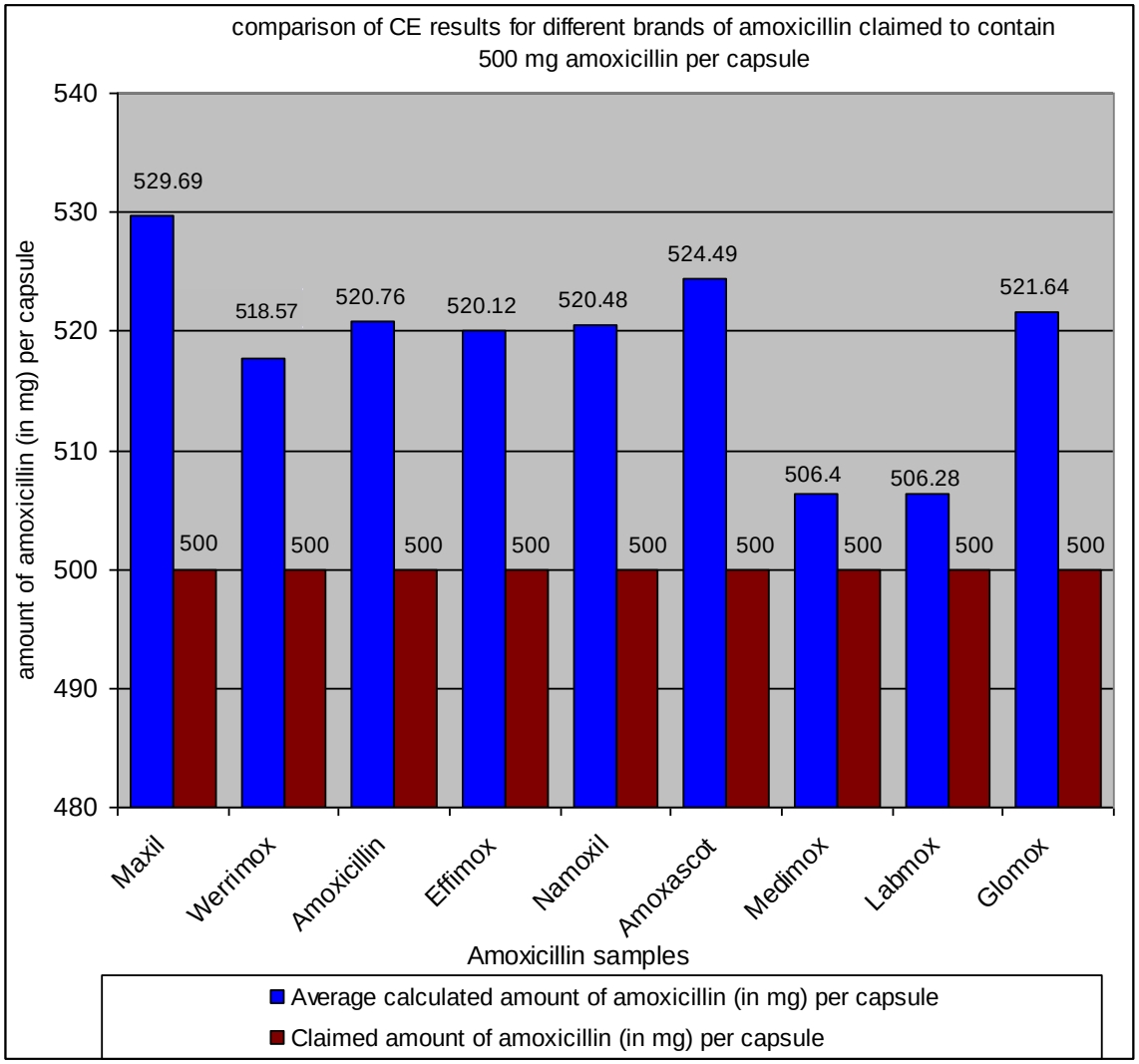


Figure.5.3.5: comparison of CE results obtained for different brands of amoxicillin samples (claimed to contain 500 mg amoxicillin per capsule). The chart suggests that almost all the samples contain excess amount of amoxicillin compared to the claimed amount of 500 mg amoxicillin per sample capsule.

Similarly the CE results for the samples claimed to contain 250 mg amoxicillin per sample capsule were compared separately and the comparison chart is given in the Figure 5.3.6 on the next page.

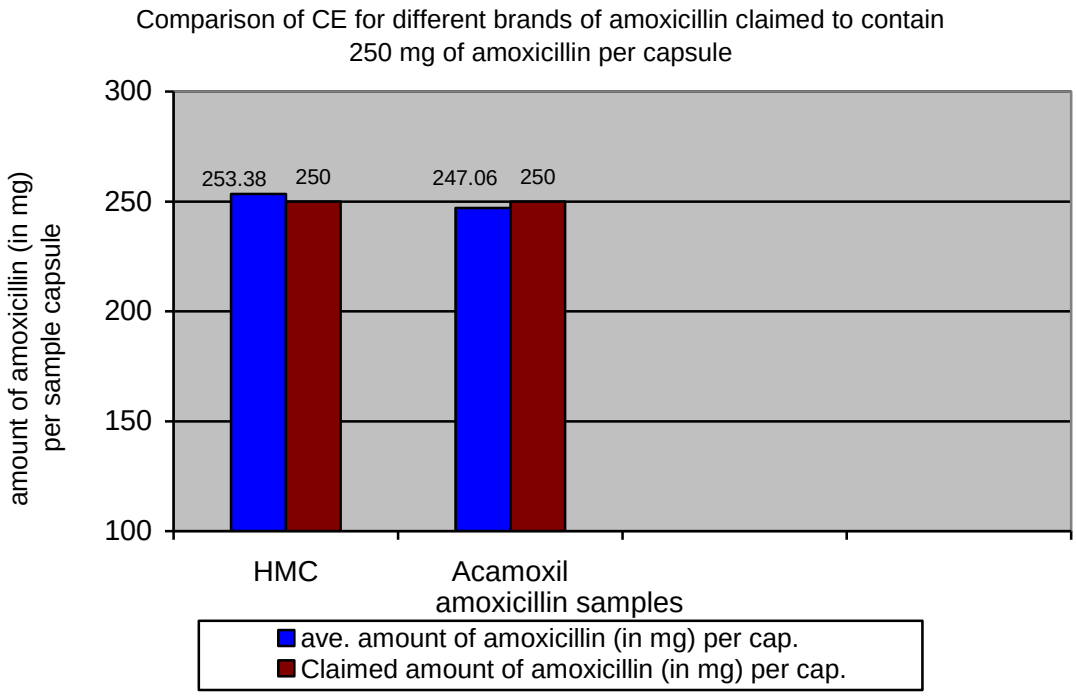
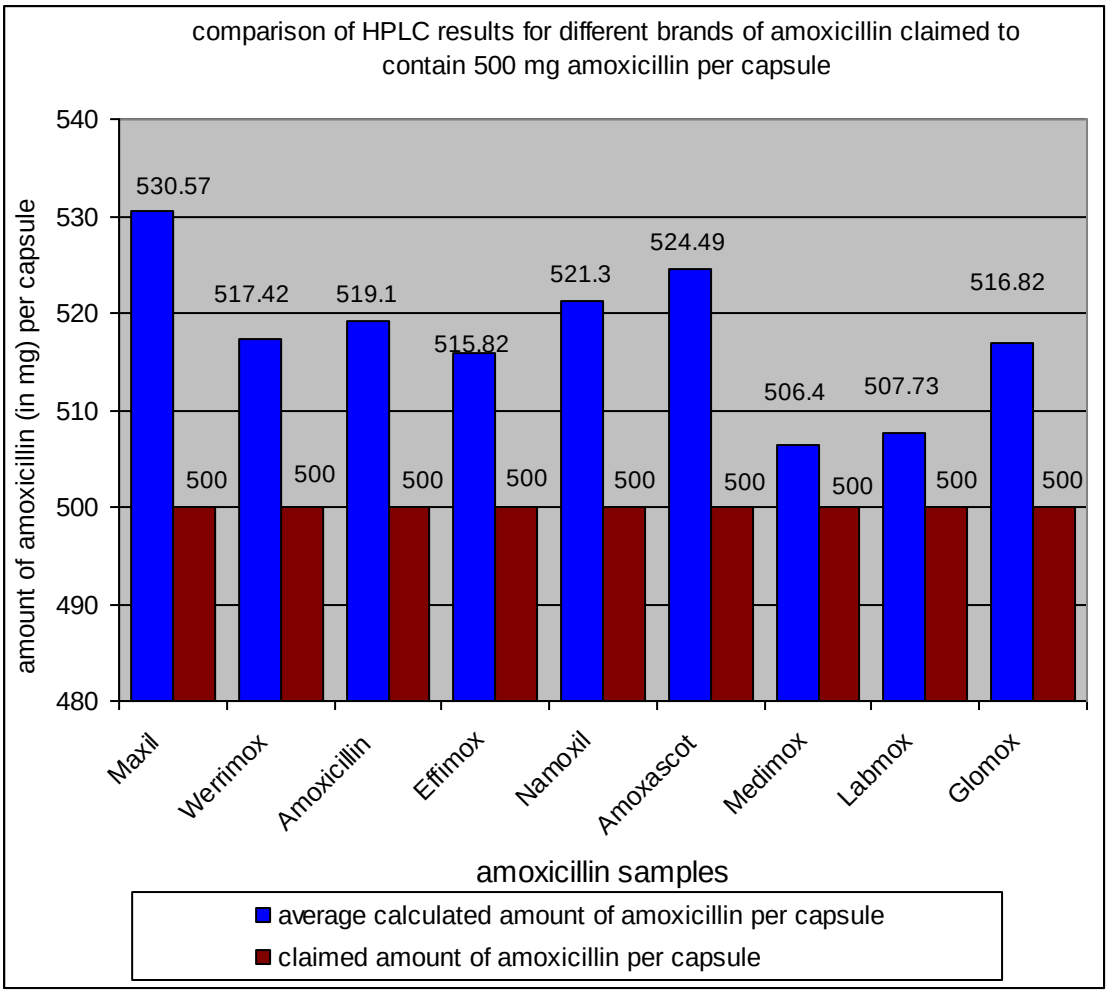


Figure.5.3.6: The chart suggests that the sample HMC shows the same average amount of amoxicillin per sample capsule as the claimed amount of 250 mg amoxicillin per sample capsule using CE method. similarly sample Acamoxil is found to contain the same average amount of amoxicillin per sample capsule as the claimed amount of 250 mg amoxicillin per sample capsule.

The HPLC results for all the samples were also compared and the values were expressed in the form of bar charts given in the Figure 5.3.7 and Figure 5.2.8 on the following pages.



Figur.5.3.7: Comparison graph showing the HPLC results for different brands of amoxicillin. The results suggest that almost all the different brands of amoxicillin are found to contain the average amount of amoxicillin per sample capsule in excess to the claimed amount of 500 mg amoxicillin per sample capsule.

The comparison of HPLC results for samples claimed to contain 250 mg amoxicillin per sample capsule is given in the form of a separate chart and is given in the Figure 5.3.8 on the following page.

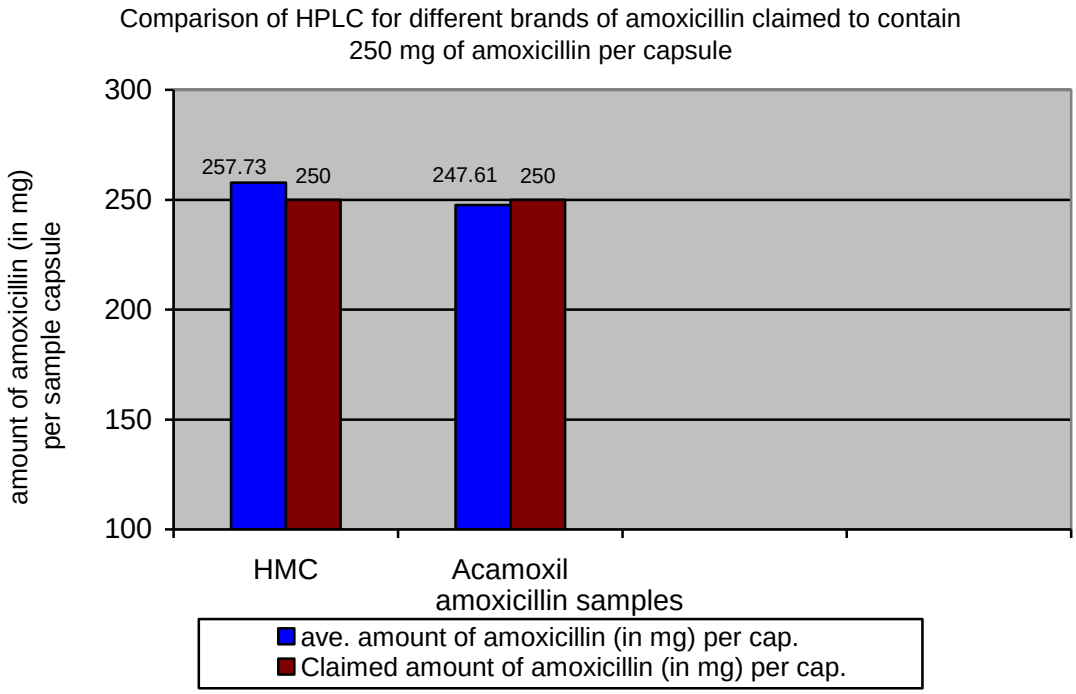


Figure.5.3.8: The chart suggests that the sample HMC shows a slightly higher average amount of amoxicillin per sample capsule compared to the claimed amount of 250 mg using HPLC method. While sample Acamoxil is found to contain nearly the same average amount of amoxicillin per sample capsule as the claimed amount of 250 mg amoxicillin per sample capsule.

All the comparison charts given above explain the quality of all the different brands of amoxicillin samples obtained from different parts of the Middle East and Subcontinent. It was found that almost all the samples contained relative amount of active ingredients (amoxicillin) and did not show any counterfeiting excluding sample 10.

Sample 10 (Acamoxil) was found to be counterfeiting as one capsule out of 4 did not contain any amoxicillin at all. There fore sample 10 (Acamoxil) was found to be counterfeit. All other samples contained excess amount of amoxicillin.

5.2: Comparison of IR Results Produced by all the Amoxicillin Samples to those Produced by Pure Amoxicillin.

The characteristic strong peaks observed in the IR spectrum of all the amoxicillin samples are compared with IR spectrum of pure amoxicillin and are listed as follows.

Table.5.1.4: Comparison of IR spectrum of all the amoxicillin samples with that of pure amoxicillin.

Sample name	λ Absorb.(max)$^{-1}$	λ Absorb.(max) $^{-2}$
Pure Amoxicillin	1686.32 cm^{-1}	1775.10 cm^{-1}
Maxil	1686.34 cm^{-1}	1775.21 cm^{-1}
Werrimox	1686.27 cm^{-1}	1775.13 cm^{-1}
Amoxicillin	1686.43 cm^{-1}	1774.97 cm^{-1}
Effimox 500 mg	1686.43 cm^{-1}	1775.15 cm^{-1}
Namoxil	1686.27 cm^{-1}	1775.13 cm^{-1}
Amoxascot	1686.29 cm^{-1}	1775.15 cm^{-1}
Medimox	1686.27 cm^{-1}	1775.13 cm^{-1}
Labmox	1686.27 cm^{-1}	1775.13 cm^{-1}
HMC	1686.43 cm^{-1}	1774.84 cm^{-1}
Acamoxil	1686.63 cm^{-1}	1775.06 cm^{-1}
Glomox	1686.27 cm^{-1}	1775.13 cm^{-1}

Similarly, all other peaks found in the IR spectra of different samples of amoxicillin were compared with IR spectrum of pure amoxicillin. It was found that all the IR spectra obtained for different samples of amoxicillin not only resemble the IR spectrum of pure amoxicillin were found similar for all of the amoxicillin samples.

The similarity of all the peaks found in IR spectra of amoxicillin samples and pure

amoxicillin confirmed the presence of amoxicillin in all of the samples analysed using IR technique. After the presence of amoxicillin was confirmed in the samples, these samples were further analysed using HPLC and Capillary Electrophoresis.

5.3: Critical Review of the Project Outcome:

Reviewing all the results obtained for all the samples, it is found that all the samples (excluding sample 10) did not show any counterfeiting. However, the samples were found to have variation in the content uniformity within the same brand but still all these samples meet the international standards of quality control.

Medicines can cause adverse effects if they are taken in excess to the amount prescribed by the physician or pharmacist. The patients are more likely to take excessive doses of the medicines having active ingredients in excess to the amount stated or mentioned by the manufacturer because the doctor or physician may prescribe the required quantity only according to the quantity mentioned by the manufacturer.

Similarly, if a medicine is a combination of two active ingredients of which one is mentioned on the label or packaging of the medicine and the other is hidden. A patient is advised to take the said medicine according to the stated ingredient. The patient can be allergic to the ingredient not mentioned by the manufacturer and thus might experience an adverse effect. Such potentially dangerous drugs can be called as unexpected pharmaceuticals (Newton. P.N, *et al*: 2006 Lancet).

A literature review shows that anti-infective drugs having little or no active ingredients may result in ineffective and even harmful treatment and can cause death in some cases (Newton. P.N, *et al*: 2006 Lancet).

Anti-infective drugs having little or no active ingredients resulting in ineffective

treatment can increase the chances of spreading the infection or disease under treatment and eventually can lead to death. Therefore, any drug having little active ingredients or having active ingredients in excess to the amount claimed or stated by the manufacturer are as harmful and fatal as any other fake drug.

As some of the amoxicillin samples analysed during this research project were found to contain higher values of amoxicillin compared to the amount stated by their respective manufacturers, but literature study reveals that all the samples meet the international quality standards of medicines i.e. a sample tablet or capsule of 500 mg should not contain less than 90 % of active ingredients of the stated amount and similarly no sample should contain more than 120 % of the stated amount (United State's Pharmacopoeia).

Summary of Chapter 5:

The 1st section of chapter 5 comprises all the results and discussion of CE and HPLC and IR analysis of all amoxicillin samples. The average values of calculated amount of amoxicillin in all the samples were compared with the claimed amount of amoxicillin per sample capsule. The HPLC results were compared with CE results for all the samples. The comparison show that the results obtained from both HPLC and CE were almost the same with some minor differences for some of the samples.

HPLC and CE results proved all the samples contained amoxicillin more or less the amount mentioned or stated by their manufacturers. All the samples were found to show a variation in content uniformity but did not show any counterfeiting excluding sample 10. The only sample was sample 10 which was found to be substandard or counterfeiting. One capsule out of four from sample 10 did not contain any amoxicillin at all and was claimed by the manufacturer to contain 500 mg of amoxicillin per sample capsule.

The IR results were discussed for all amoxicillin samples. The IR spectra of all

the samples were compared to IR spectrum of pure amoxicillin. The IR results for all amoxicillin samples were the same as for pure amoxicillin confirming the presence of amoxicillin in all samples.

Chapter 6: "conclusion and future study"

This research project is aimed to study amoxicillin in counterfeit antibiotics from the Middle East and Subcontinent. These antibiotics were of different brands and were obtained from different parts of the Middle East and Subcontinent such as Iraq, China, India and Pakistan. The samples collected randomly from open market manufactured different companies of these different regions were anlysed quantitatively using the modern analytical techniques such as High Performance Liquid Chromatography and Capillary Electrophoresis and the results thus obtained were then compared for quality purpose to find counterfeiting. The project was started with the development of a suitable CE method for the analysis of amoxicillin and then further research work proceeded with the use of HPLC and IR.

Conclusion:

The literature review and extensive study on counterfeit drugs provided useful informations on counterfeiting around the world. The valuable research work previously done on counterfeiting by many research workers greatly highlights and explains the threat of counterfeiting in its all types and affirms that counterfeiting has become a major issue in poor and developing countries.

The research work undertaken in this project was aimed on the analysis of amoxicillin containing antibiotics obtained from the open market of Middle East and Subcontinent. All the samples were randomly obtained without any discrimination or specification. The results produced by different brands of amoxicillin confirmed that out of 11 different amoxicillin samples, 10 samples did not show any counterfeiting and contained amoxicillin more or less the amount claimed by the manufacturers which meet the international standards of quality control.

Eleven samples of amoxicillin from different countries of the Middle East and subcontinent were quantitatively analysed. It was found that all the samples

shown repeatability that is when an individual sample capsule from a particular brand was analysed several times, the results obtained after every single analysis were the same. At the same time it was found that the different capsules analysed from a particular brand did not contain the same amount of amoxicillin and a variation in content uniformity was found in all the samples. The average values of HPLC and CE results for all the different samples are summarised and listed in the Table 6.

Table.6: list of average values of HPLC and CE results for all the different samples.

Sample	HPLC results (mg)	CE results (mg)	Amount stated (mg)	Average (mg)	STDEV ±
Maxil	530.57	529.69	500	530.13	0.11
Werrimox	517.42	518.57	500	517.99	0.57
Amoxicillin	519.16	520.76	500	519.96	0.8
Effimox	515.82	520.12	500	517.97	2.15
Namoxil	521.3	520.48	500	520.89	0.41
Amoxascot	524.49	524.49	500	524.49	0
Medimox	506.4	506.4	500	506.4	0
Labmox	507.73	506.28	500	507.0	0.72
Glomox	516.82	521.64	500	519.23	2.41
HMC	257.73	253.38	250	255.56	2.17
Acamoxil	185.71	185.29	250	185.5	0.21

All the results listed in the Table 6 were obtained from the experimental work undertaken during this project lead to a conclusion that all the different brands of amoxicillin (excluding sample 10: Acamoxil obtained from Iraq) contained amoxicillin more or less equal to the value claimed by their manufacturer and meet the international standards of quality control thus did not show any counterfeiting.

Table 6 also shows that there is a difference in the HPLC and CE results. The

reason for this difference has not been investigated at this level of study. Furthermore, this difference appears not to be because of either HPLC or CE as the retention and migration time of amoxicillin samples recorded from HPLC analysis and CE analysis respectively is within the limits as found in the literature proving the instruments were accurate.

Furthermore, sample 10 which was obtained from Iraq, was found to be counterfeit as one sample capsule (capsule 10 a) did not contain any amoxicillin at all. These results were confirmed after a repeated analysis of sample 10 a by HPLC and CE techniques. None of these two techniques confirmed the presence of any amoxicillin in the sample capsule 10 a.

As other three capsules analysed from sample 10 produced good results and the quality standards were found to be within the limits of international standards of quality control. Hence, it is concluded that the manufacturer might have produced this drug having the quality standards which meet the international standards of quality control and that the counterfeiting might have come from any point along the distribution chain.

One of the possibilities for this counterfeiting is that one of the original capsules might have been replaced with a capsule having no amoxicillin and the original product might have been repacked. As only four capsules out of ten from sample 10 were analysed and one capsule was found counterfeit, there is the possibility of more counterfeit capsules in that pack of 10 capsules of sample 10 .i.e. more than one of the original capsules might have been replaced with the empty or inert ones.

Future study:

This project enabled the analysis of antibiotics using capillary electrophoresis and High Performance Liquid Chromatography under different experimental conditions helping the development of a suitable method on CE and HPLC for further project work. Further research work may help developing more easy and simple methods

of HPLC and CE analysis in the future to study not only counterfeit antibiotics but other counterfeit drugs as well.

Further analysis could be carried out by keeping the antibiotic stock solution for several days and then analyse them on HPLC and CE. This will enable to find the difference between the results obtained from old stock solutions and those obtained from freshly prepared solutions.

This project not only enables for further analysis of other antibiotics from Middle East and different countries from Asia such as Iran, Turkey, Nepal, Bangladesh, Afghanistan, Bhutan and Sri Lanka but will also facilitate the analysis of other counterfeit drugs in the developing countries.

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